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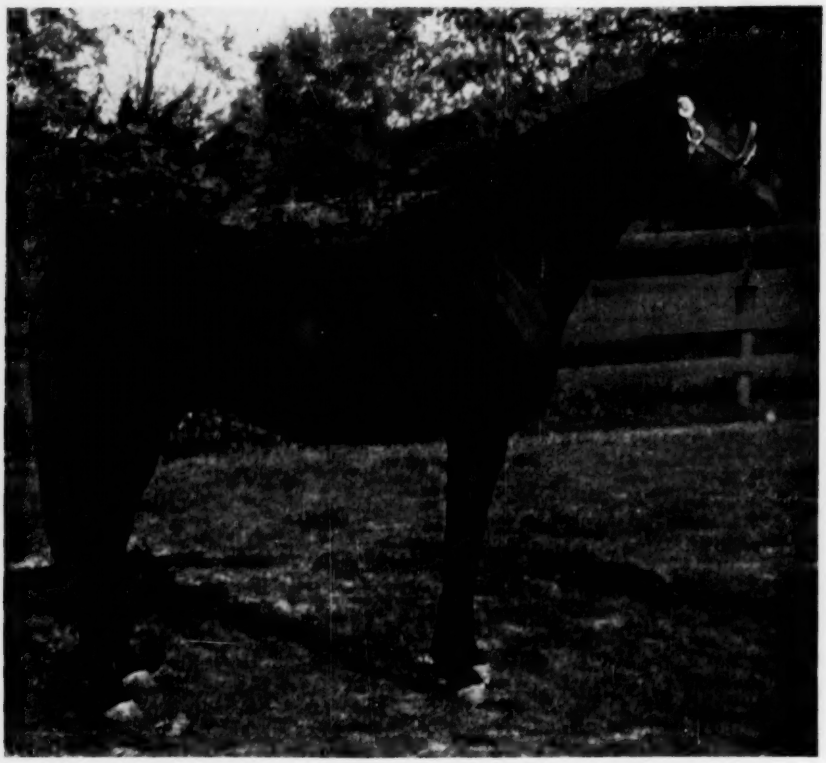

"BROWN BEAUTY"—AN INTERMEDIATE GENOTYPE
(See Page 127)

Origin of Johnson Grass

Light Undercolor in the Hamster

Studies on the Mouse Hemoglobin Locus

VOLUME 51 MAY-JUNE, 1960 NUMBER 3

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

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Figure 1

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ORIGIN OF JOHNSON GRASS

A. Ghafoor Bhatti, John E. Endrizzi and R. G. Reeves*

THE origin of Johnson grass (*Sorghum halepense* Pers.) has been the subject of many cytological and genetical studies, and a number of attempts have been made to ascertain the relationship between *S. halepense* and other species in the section *Eu-sorghum*. The cytotaxonomic work done in this connection has been reviewed recently by Endrizzi[3] and Celarier[2]. The consensus seems to be that *S. halepense* ($2n = 40$) was evolved by doubling the chromosomes of a hybrid between *S. vulgare* Pers. and some related 20-chromosome species. However, none of the 20-chromosome species of *Sorghum* was known to have rhizomes except *S. propinquum* Hitchc., which has been reported only from the Philippines and southeastern Asia, areas very remote from the Mediterranean region where *S. halepense* is native. Celarier[2] expressed the opinion that since none of the African species of *Sorghum* have rhizomes, *S. halepense* might have originated in Indochina and Burma as a result of a cross between *S. propinquum* and a local unknown 20-chromosome species, followed by a doubling of the chromosomes.

Endrizzi[3], on the other hand, considered *S. propinquum* to have originated as a polyhaploid of *S. halepense*. He supported his hypothesis by a report of a polyhaploid obtained from *S. halepense* by the Indian workers Raman and Krishnaswamy[7]. This polyhaploid had broader leaves and larger stems than *S. halepense* and had normal fertility. The previously known 20-chromosome forms of *S. halepense* can be readily crossed with *S. vulgare*, yielding fertile hybrids with normal meiosis. The description of the polyhaploid shows that it resembled *S. propinquum* so closely that Endrizzi felt justified in his hypothesis, but the question of the origin of rhizomes in *S. halepense* still remained unsolved.

During the course of a study of the cytogenetic relationship of the two 20-chromosome species *S. vulgare* and *S. virgatum* Stapf, a tetraploid plant was obtained in the summer of 1958 by doubling the chromosome number of the F_1 hybrid between *S. vulgare* and *S. virgatum*. This plant was moved into the greenhouse in October 1958, and selfed seeds were obtained in January and February. In late February about 400 of these seeds were planted in cups in the greenhouse, and later, when the seedlings were eight to 10 inches tall, they were transplanted into the field. Plants were spaced 18 inches apart in 40-inch rows. All the plants made normal growth, and there was no great difference in general growth habit of the plants until the flowering stage. It was then observed that two of them were strongly rhizomatic (Figure 1 *E* and *F*) and closely resembled *S. halepense* in general appearance, leaf shape, stem diameter, size of the spikelets, and shape of the panicle (Figure 2 *I* and *K*). The fact that the seeds of the tetraploid *vulgare-virgatum* hybrid were produced in the winter in the greenhouse, where there were no plants of *S. halepense*, eliminated the possibility of any cross-pollination with *S. halepense*, and the planting of individual seeds in cups minimized the chances of mechanical mixture. Yet a decision as to whether these Johnson grass-like plants were actually segregates of the cross depended in part on whether some of the F_2 plants of the population formed intermediate rhizomes, i.e., rhizomes much weaker than those of the Johnson grass-like plants.

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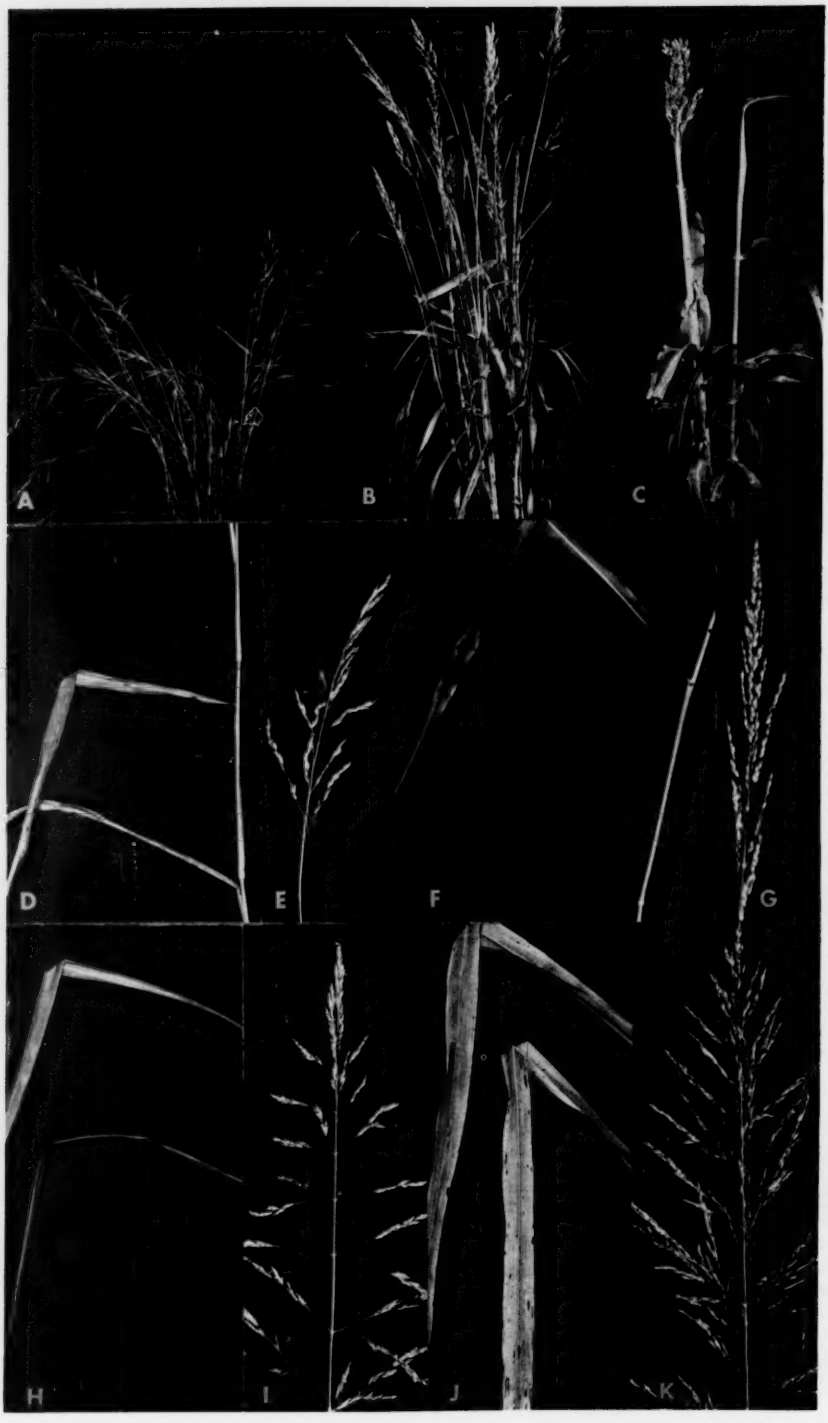
A
B
C
D
E
F
G
H
I
J
K

On careful study of the tetraploid progeny, it was found that several other plants did have weakly developed rhizomes (Figure 1 *C* and *D*). These plants resembled the rest of the population in general appearance and panicle characters, and had rhizomes similar to those reported by Hadley and Mahan[6] and Hadley[4] in crosses between Johnson grass and *S. vulgare*.

The occurrence of rhizomes in the 40-chromosome derivatives of *vulgare-virgatum* hybrids appeared paradoxical because neither of the parents was known to have rhizomes. Upon close examination, however, *S. virgatum* was in fact observed to have a few rhizomes, which were so small that they could be easily overlooked on casual examination (Figure 1 *A* and *B*).

The occurrence of strong rhizomes on segregates of the tetraploid *vulgare-virgatum* hybrid is explainable on a principle which is becoming widely recognized. Stebbins[8], in a review of the literature, has shown evidence that although the doubling of the chromosome number of typical annuals results only in annual polyploids, doubling of the number in annuals having perennial tendencies often results in strong perennials. In the plants under study here, the weak rhizomatic character of *S. virgatum* supplies the perennial tendency, which is augmented in the tetraploid offspring.

Sorghum virgatum has a strong probability of being the 20-chromosome species which in combination with *S. vulgare* gave rise to *S. halepense* at the tetraploid level. *S. virgatum* is not only native to the same area that is considered to be the place of origin of *S. halepense*, but it also could have contributed the grassy habit, the deciduous spikelets, and the rhizomatic tendencies.

The cytological behavior of the tetraploid derivatives of these *vulgare-virgatum* hybrids is the same as that reported for *S. halepense*. The average frequency of chromosomal pairing at metaphase I observed in the 40-chromosome *vulgare-virgatum* hybrids, as compared with that reported for *S. halepense*, is given in Table I.

Furthermore, in the progeny of the tetraploid *vulgare-virgatum* hybrid, the genes ms_2 (male sterility) and *d* (dull midrib) segregated in 35:1 and 21:1 ratios of dominant and recessive classes, which is in agreement with the hypothesis of random chromosome pairing. This is in agreement also with results reported by Casady and Anderson[1] in their crosses between tetraploid *Sorghum sudanense* (Piper) Stapf and *S. halepense*. Similarity of chromosomes of *S. vulgare* and *S. virgatum*, as indicated by normal pairing in the diploid F_1 hybrid, is in agreement with the conclusion drawn by Endrizzi[3] from his cytological studies of the hybrids between *S. halepense* and *S. vulgare*, that the two genomes of *S. halepense* Pers. are similar to each other.

The foregoing cytological and geneti-

TABLE I. Chromosomal pairing at metaphase I in Johnson grass and 4*n* *vulgare-virgatum* hybrid

Type	No. of cells	Av. chromosome association per cell IV	III	II	I
Johnson grass*	148	2.85	.15	13.92	.56
4*n* *vulgare-virgatum* hybrid	105	2.9	0.0	14.18	.04

*Average of the results reported by Hadley[5], Endrizzi[3].

LEAF AND PANICLE COMPARISONS

Figure 2

A—*Sorghum virgatum*. *B*—4*n* *vulgare-virgatum* hybrid, intermediate between its parents. *C*—*S. vulgare*. *D* and *E*—Leaf and panicle of *S. virgatum*. *F* and *G*—Leaf and panicle of 4*n* *vulgare-virgatum* hybrid with intermediate rhizome expression. *H* and *I*—Leaf and panicle of 4*n* *vulgare-virgatum* hybrid with extreme rhizome expression. *J* and *K*—Leaf and panicle of *S. halepense*.

cal evidence supports the conclusion that Johnson grass arose as a result of the doubling of chromosomes of a natural hybrid between *S. vulgare* and *S. virgatum* in the Mediterranean region of North Africa.

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◆

THE KURU DISEASE IN NEW GUINEA

THE July 8 issue of *Science* carried a note by Dr. Theodosius Dobzhansky (here reprinted by permission) regarding a remarkable genetic situation in a New Guinea tribe. The control of the so-called "laughing disease" marks an unusual departure in governmental eugenic policy.

"A plan to introduce a eugenic policy, apparently without precedent anywhere, has been announced by the administration of the Territory of New Guinea [*South Pacific Post* (24 May 1960)]. This measure affects the tribe of Fore, some 30,000 strong, which inhabits an area of about 884 square miles in the Eastern Highlands. Almost half of the women and one-tenth of the men of the Fore tribe die of a hereditary disease known as kuru. This is a grave neuropathological disturbance for which no cure is known and which leads to death, usually within a few months from the appearance of the first symptoms.

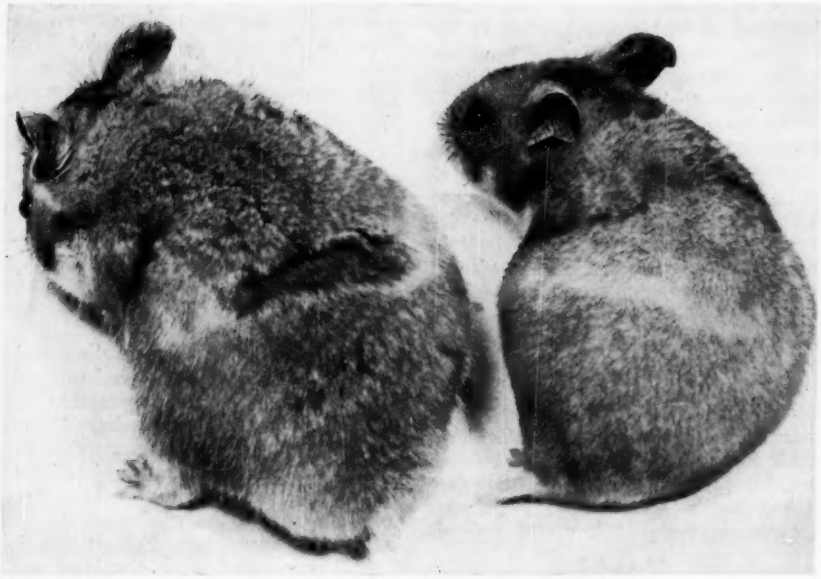
"As shown by studies conducted in the Fore area by a group of investigators from the University of Adelaide (South Australia), kuru is transmitted by a gene which behaves apparently as a Mendelian dominant in females and a recessive in males. The presumed homozygotes of both sexes die in childhood, usually before adolescence; heterozygous females die later, after most of them have produced children. The extremely high incidence of kuru in the Fore tribe and its absence in neighboring tribes except as introduced by migrants from the Fore area remain unexplained. The heterozygous carriers of the gene for kuru probably possess some considerable adaptive advantage, both the males who survive and perhaps also the females who die after having completed at least a part of their reproductive lives. The nature of this advantage is, however, completely unknown.

"In part because of the ravages of kuru, some portions of the Fore tribe have great excesses of males over females (as much as 2.5 males per female). Fore men tend to move into neighboring tribes, and some of them, like members of tribes in which kuru is unknown, come as contract laborers to work on plantations in other parts of New Guinea. Since many of them are heterozygous carriers of the gene for kuru, it is feared that the disease may spread and afflict the populations of other districts.

"To counteract this danger, the administration's plan is to quarantine the Fore tribe, by prohibiting the emigration of its members from the tribal area. All Fore men who have been recruited as laborers from the area will be returned there. This is obviously a severe restriction to be imposed on a whole tribe. Realizing this, the administration is considering ways and means of developing the Fore area to provide work for its people and to make emigration unnecessary. The study of the disease is to be continued, in the hope of finding a remedy for kuru victims and a way of identifying the carriers of the kuru gene in heterozygous condition before the appearance of the disease symptoms."

LIGHT UNDERCOLOR IN THE SYRIAN HAMSTER

ROY ROBINSON*



NORMAL AND LIGHT UNDERCOLOR

Figure 3

On the left is a normal hamster with dark blue undercolor and on the right a light hamster with white undercolor.

DURING the course of studies on the simultaneous segregation of several mutant genes in the Syrian hamster (*Mesocricetus auratus*), a number of curiously colored individuals came under observation. These variants consisted of three golden agouti animals with very light or whitish undercolor. The coat pigmentation of the normal hamster has been described in detail by Robinson[9]. Normally, the undercolor (defined as the eumelanic pigment basal to that of the yellow agouti band on the hair fibers) of the dorsal hairs is deep blue, paling to grey towards the roots.

In the variants, however, the undercolor varied from light blue to grey-white, the color varying in this manner generally from the shoulder to the rump. The animals also varied among themselves. One of the three (the largest of the trio) possessed perceptibly deeper undercolor, a pale blue. The light undercolor was most prominent in the full juvenile coat of about three to four weeks of age. Some darkening was evident in the succeeding intermediate coat

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and, as adults, the coat color was almost typically agouti hamster. However, this was not completely so; a small reduction in the over-all intensity of yellow and a paler undercolor could be discerned, in comparison with the normal. The eye color did not appear to be affected at any stage of development. This variation from normal coloration occurs infrequently among colonies of hamsters but is sufficiently distinctive to stimulate comment when it does occur.

The three variants were males; one was found to be sterile and one had to be relinquished to fancy breeding. It may be stated briefly that no records came to light upon enquiry that any of the descendants of this male exhibited the light undercolor. The third male was subjected to extensive breeding tests. The objects of these were: (1) to determine if the variant color was inherited and, this being the case, (2) in which manner, and (3) to discover the possible relationships of the color with other known mutant color phenotypes. The evidence tends to indicate either that the color is not due primarily to genetic causes or, if so, that a special genetic situation must supervene.

Material

The breeding material consisted of the variant male crossed with females homozygous for two recessive mutant genes, and particularly, the F_2 and F_3 generations of this cross, together with a few incidental observations. The mutant genes involved have been described fully by Robinson[9]. In summary, these are non-extension of black (*e*), which produces a cream coat color, and a ruby eye dilution (*ru*), which dilutes both eye and coat color. Those animals with the light undercolor will, for convenience, be referred to as "lights."

Results

The original variant male was mated with females of genotype *eeruru*, producing 38 wild-type agouti; and with two wild-type females, producing nine offspring, also wild type. The total of 47 normal agouti almost certainly disposes of the hypothesis that the variant phenotype is due to heterozygosity of a fully penetrant dominant gene.

The F_2 from the mating with *eeruru* produced the following distribution:

++	e+	+ru	eru	Total
196	60	33	7	296

None of the mutant phenotypes displayed a deviation of undercolor from that usually expected. Of the 196 normals, however, all were normal except for seven offspring from one female (16c) which possessed light undercolor. The undercolor in these exceptional animals was lighter than normal but darker than observed in the original animals. These figures disprove that the light phenotype is due to a regularly manifesting recessive gene. The possibility of irregular manifestation is considered below.

The breeding behavior of the female 16c may be arranged as Table I to facilitate analysis. It can be seen that the light animals were born in later litters, which prompted the inference that the undercolor could be due to a deteriorating uterine environment or to a progressively increasing deficiency in the milk from the mother. To test this the female was mated to an unrelated normal male for her fifth litter. The outcome, however, was a litter of six normals, all having dark undercolor.

A second suggestion is indicated by the fact that the two litters in which the "lights" appeared were sired by the same male, but a different male from those siring the first couple. Although the numbers were regrettably few in the first two litters, this could indicate a genetic influence. It could be that the original variant male was not genetically homozygous for light phenotype

TABLE I. Breeding record of female 16c

Litter	Male	Agouti		Mutant phenotypes
		Normal	Light	
1	9a	2	0	2
2	9b	2	0	3
3	15b	3	2	2
4	15b	0	5	3
5	58a	6	0	0

engendering genes (if such exist) and the appearance of these would depend upon the chance mating of F_1 animals carrying certain combinations of genes. Henceforth, male 15b was preferentially utilized for further matings and produced 104 offspring from various F_1 females, but all of these were normal. The F_1 generation was composed of four males and 18 females and, if the immediate suggestion is to be sustained, it must be assumed that among these only animals 16c and 15b received gene combinations which subsequently re-formed in the F_2 to engender a light phenotype. Fifteen of the F_1 females had at least one litter by male 15b.

Both the possibilities, of non-manifestation of a recessive gene and the event of a rare gene combination, may be tested by breeding from the light animals recovered in the F_2. "Lights" from the two litters were mated together, producing 33 offspring, all with dark undercolor. The number in this F_2 is unfortunately small, but the data imply that if impenetrance of a single gene is involved the penetrance must be low. In the event of rare combination of genes, the data imply heterozygosity of the F_2 parents. This is not impossible but, if true, it would seem that the genetic situation must be complex.

The adult coat of light animals shows an over-all lightening, particularly in the yellow sub-apical hair pigment and in the undercolor. Such a phenotype could be due to a "chinchilla" type of dilution, which (1) has the white undercolor as a side-effect of the gene's main action and (2) overlaps phenotypically with the wild type. Feldman[1] has described an "intense chinchilla" in the house mouse with two of these properties, namely, some confusion with normal and a small reduction in intensity of yellow. Chinchilla alleles of albinism are often incompletely dominant to the lower alleles. If light is a chinchilla-type allele, and incompletely dominant to the known albinotic allele, c^d, of the hamster (see Robinson[10] for details of this mutant), it may be possible to make the light phenotype more pronounced by crossing to c^dc^d. This has been accomplished; two of the F_2 "lights," mated to c^dc^d, gave 17 young, each of which, however, was normal in appearance. No support is thus forthcoming that the light phenotype behaves as a typical chinchilla allele.

An ancillary item from the F_2 distribution is the feasibility of a phenotypic interaction of light with the ruby-eye coat color. The ruby eye gene dilutes both yellow and black, the yellow to fawn and the black to bluish, with a corresponding dilution of the undercolor. Should light and ruby eye interact to enhance the effect of one or the other, light animals may be more readily distinguished from non-lights, but no phenomenon of this nature has been observed in the F_2. The shortage of *ruru* individuals to be noted in the F_2 distribution is not unexpected. Animals homozygous for *ru* are of impaired viability and exposed to excessive prenatal mortality[9]. The present F_2 forms part of that reported as cross I in the 1958 paper, where these various aspects are fully discussed.

Contemporaneously to the F_2 detailed above, it was noticed that a pair of animals were producing light young. The litters from this pair are presented in Table II. The pair are sibs and are collaterally related to the variant males, but at a distance of at least three generations. Female 59d has been paired only with the male 59a, but the male has also been mated to normal unrelated females. The offspring consisted of 13 agouti and 12 mutants, all with normal undercolor. Immediately the light young were noticed, the male was mated to three of the F_1 females from the variant male. The offspring from these consisted of 18 agouti and 12 mutant phenotypes, but all of these were perfectly normal as

TABLE II. Breeding record of female 59d

Litter	Male	Agouti		Mutant phenotypes
		Normal	Light	
1	59a	5	0	0
2	59a	4	1	3
3	59a	1	2	3

regards undercolor. The one interesting feature contributed by this pair is that the "lights" occurred in the later litters as a rising proportion of the agouti (non-mutant) class. This is similar behavior to the female of Table I and may yet be of significance.

Discussion

The possible effects of several environmental factors were constant over the entire stock of animals, with the exception of the early life of the original variant males. These included diet, temperature, humidity, and intensity and duration of illumination. The last four items were subject to normal seasonal variation but constant at any time. At any instant, in the course of the experiment, there were several dozen maturing young hamsters exposed to these influences. In view of the absence of any general appearance of light undercolor, it is considered that the effects of these are negligible. The environment to which the original males were exposed prior to their examination was unknown but it was stated by their breeder that this was not exceptional. Sex is not a factor to be considered since, aside from the original three males, the 13 other anomalously colored animals consisted of seven males and six females.

The perplexing feature of the breeding results obtained with the light undercolor character is the apparently weak heritability component in its incidence. In other words, while a genetic disposition may underlie the low incidence, either the present material is insufficient to bring this out clearly or the genetic influence is too nebulous for a simple explanation to be offered. The simple hypothesis of a recessive gene with very low penetrance could be fitted to the data but not with any worthwhile assurance. A more probable explanation is that of a polygenic complex of genotypes, some of which cross an ontogenic threshold resulting in a temporary failure of pigment formation. Alternatively to an ontogenic threshold, a uterinal or lactational influence could provide the required trigger mechanism. It is unfortunate that no auxiliary information is in existence to support the explanation, as for the case to be cited in the rabbit.

It is of interest that accounts of white undercolor in various species of rodents may be found in the literature. Some of these have been shown either not to be inherited or to be feebly inherited, while others are relatively simple monogenic characters. The observations of Huestis[1] are in closest accord with the present results, in spite of the fact that these refer to a loss of pigment in the distal, rather than in the proximal, portions of the hairs relating to the skin.

"Light" or "pale" pelages have been observed in *Peromyscus*[2, 5]. The pale pelage is due to absence of pigment in the distal part of the hairs. The pale coat may succeed a normally pigmented coat or itself be replaced by a normal coat. Morgan merely presents a description but Huestis gives some indefinite breeding results. Two pale animals mated *inter se* yielded four young, three of which were pale and one slightly pale in the post-juvenile coat. Unfortunately, this family was accidentally killed. A pale male mated to two half-sisters (not stated if pale) yielded 10 young, one of which, a female, was partially pale in the post-juvenile coat. This daughter and two others were mated with the male, produced 17 young, none of which were pale. Examination of the breeding of other relatives of the pale male, and of additional animals with pale pelages, induced the comment by Huestis: "These data, without being genetically critical, add to the probability that a familial tendency to grow a light pelage is not very pronounced in the environment in which I kept the stock."

A white undercolor, which is present in the juvenile coat but not in subsequent coats, is known in the rabbit[6, 8]. The inheritance of the undercolor is not understood but its causation can be surmised. The undercolor arises in blue diluted (*dd*) breeds in which fanciers have selected for extremely light shades, and so far as is known, only in this type of animal. It could be due to a temporary failure of pigment formation, and an obvious provisional explanation would be that of a polygenic character manifesting whenever an ontogenic threshold is passed. A similar mechanism may be operating in the hamster, although the case for its acceptance is not as convincing as may be desired (the absence, for example, of any known prior selection).

A semi-dominant gene which resembles the light hamster in some respects, especially in its extreme expression, has been described in guinea-pigs by Ibsen and Goertzen[3] and termed "whitish." The expression of the gene varies considerably and less extreme animals display bands of depigmentation on the main hair shaft, rather than complete loss. The manifestation in black guinea-pigs is erratic and some overlapping with normal occurs, with an accompanying decrease in expression with age. In browns this age decrease is sex differentiated, the male tending to retain the whitish coloration. The gene is hypostatic to yellow and several of the albino alleles. A closer resemblance may be found in the non-agouti light house mouse[4] ($aaB^{lt}B^{lt}$) and to a lesser extent in the agouti light[7] ($AaB^{lt}B^{lt}$), since the yellow sub-apical band is not affected by B^{lt}. In the hamster, on the other hand, both yellow and black appear to be affected, at least in the golden (agouti). Another departure is that the light mouse becomes progressively lighter with advancing age. In the mouse, the light undercolor is

produced by a dominant gene, either allelic to *B* or very closely linked to it. In consequence of the dominant monogenic inheritance of the trait in both of these rodents, neither can offer, by analogy, an explanation for the hamster.

Summary

An extremely pale or whitish undercolor is described in the hamster. This occurs infrequently and sporadically among young animals in the juvenile pelage. In succeeding pelages, the animal may assume normal or almost normal appearance. In many of these animals, however, the color may appear diluted, with some reduction in the intensity of yellow and a pale undercolor. Breeding experiments indicate that the condition may be familial and, as such, have a genetic basis.

The results are too indecisive to permit of firm conclusions on the nature of this.

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A FURTHER MUTATION OF THE MOTTLED TYPE

In the House Mouse

MARY F. LYON*

THE sex-linked mutants mottled, *Mo*, and brindled, Mo^{br}, of the house mouse were reported by Fraser, Sobey and Spicer[5]. Brindled has been shown to be allelic with mottled[4] but differs from it in that male brindled hemizygotes live to about two weeks after birth whereas mottled hemizygotes die as embryos at about 12 days' gestation[2]. Since these two alleles were found there have been at least three other reports of similar mutants. Dappled, *Dp*,[6] is probably allelic with the first two as it shows approximately the same recombination as *Mo* with the sex-linked gene tabby, *Ta*, and interacts with brindled to give female compounds which resemble brindled hemizygotes. It differs from both *Mo* and Mo^{br} in that male hemizygotes die at about the time of birth. Tortoiseshell, *To*,[1] is also sex-linked, heterozygous females resembling mottled females and males being presumed to die *in utero*, so that *To* may be expected to be allelic with the other members of this complex, although no tests of allelism have been reported. Similarly, a mutant originally referred to as white-mottled and now named 26K[7] "is, judging from the results of linkage tests with *tabby*, probably an allele of *Mo*, which it resembles phenotypically."[7]

The present paper describes yet another mutation of this type. The provisional name dappled-2 was given to it, but since it is not clearly distinguishable from mottled, *Mo*, this provisional name will be withdrawn and no final name given.

Origin and Genetics of Dappled-2

The original mutant animal was a male, which was found among the offspring of the control series of a mutagenesis experiment. Phenotypically this animal resembled his later female descendants. His coat was mottled with patches of white, light-colored and full-colored hairs, and also with intermingled hairs of different colors, and his vibrissae were curly. He was mated to several normal females and sired 187 offspring, 95 normal males and 92 females of which 19 were dappled like himself. His dappled offspring when mated to normal males produced dappled females, normal females and normal males in approximately equal numbers, while his normal offspring produced only normal young (Table I).

The interpretation placed on this was that the dappled phenotype was the heterozygous expression of a gene carried on the X-chromosome, and that hemizygous males died *in utero*. The original male must have been a mosaic, with some cells, both of his soma and of his gonads, carrying a normal X-chromosome and some with a mutant X. The first dappled mutation arose in a similar way[6].

To confirm the hypothesis of sex-linkage dappled-2 was tested for linkage with tabby, *Ta*. Dappled-2 females were mated to a hemizygous *Ta* male and their *Dp-2* + *Ta* + female offspring were crossed with normal males. The results (Table II) indicate close linkage between dappled-2 and *Ta*, showing that the gene for dappled is sex-linked.

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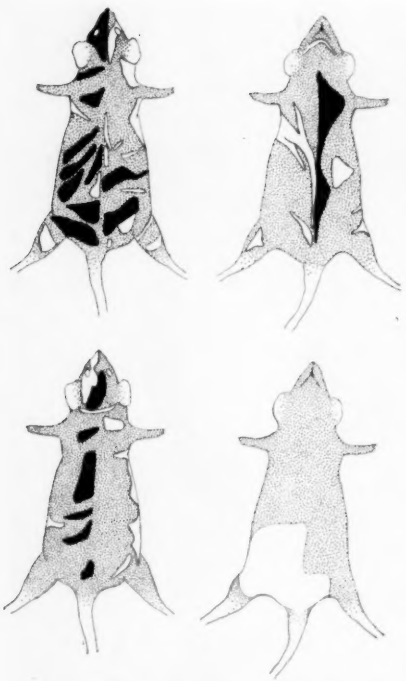


COAT COLOR PATTERNS

Figure 4

Diagrams show the dorsal and ventral sides of two typical dappled-2 females. The black areas denote full-colored and the stippled areas light-colored patches. Note that patches rarely cross the mid-line.

Relationship with the Mottled Locus

As the new mutant was sex-linked and phenotypically resembled the mottled series, it was desirable to try to establish whether it was allelic with *Mo* and, if so, whether it was distinguishable from the alleles previously reported.

The recombination between *Mo* and *Ta* is four percent[2,3] and that between dappled-2 and *Ta* is 1/17 or 5.9 percent (Table II). This is consistent with dapled-2 and *Mo* being located at the same point on the chromosome, but of course also consistent with other positions of dappled.

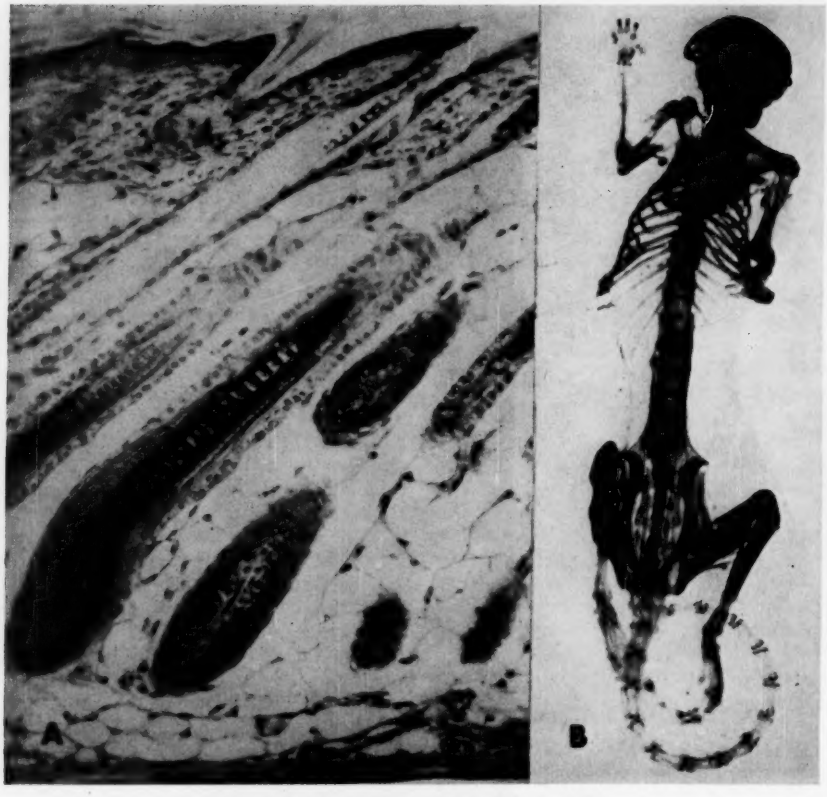
In the ordinary way it is not possible to test the various alleles, or possible alleles, of the mottled series against each other, as no males are available. A few brindled males, Mo^{br}, have survived to adulthood and become fertile, however, and one of these was kindly supplied by Dr. D. S. Falconer. When crossed with dappled females this male sired only one litter, one female of which was a black-eyed white animal and died when only 11 days old. If brindled and dappled are allelic one might expect to find females of this type which would represent the brindled-dappled compounds, so this evidence is consistent with dappled-2 being allelic with mottled, but it does not constitute proof of allelism since brindled and dappled might still interact although non-allelic.

TABLE I. Single factor segregation of dappled-2

Parents		Offspring				
		♀		♂		
♀	♂	Dappled	Normal	Dappled	Normal	Total
++	dappled	19	73	—	95	187
dappled	+	116	146	—	156	418
non-dappled	+	—	22	—	38	60
++	non-dappled	—	28	—	26	54

TABLE II. Linkage test between dappled-2 and tabby

Parents		Offspring				
		♀			♂	
♀	♂	Dappled	Tabby	Normal	Tabby	Normal
Dp-2 +/+*Ta*	++	9	9	1	7	—



ANOMALIES IN DAPPLED-2 FEMALES

Figure 5

A—Section of skin of a seven-day-old dappled female, showing two adjacent hair follicles, one fully pigmented and the other having pigment cells but no pigment granules. *B*—Alizarin-stained transparency of the skeleton of a dappled female showing many calcified lumps, in particular near the vertebrae.

Thus no one piece of evidence is sufficiently strong on its own, but taking the three available pieces of evidence together (the phenotypic resemblance of heterozygous females, position on the chromosome, and interaction with brindled), it seems the reasonable and most simple hypothesis to postulate that dappled-2 and mottled are not at distinct loci and are either allelic or pseudo-allelic.

The next question to be asked was whether dappled-2 was distinguishable from previously known alleles. In comparing the various alleles one may consider the phenotype of both the heterozygous females and the hemizygous males.

Heterozygous Females

The characteristic feature of the heterozygous dappled-2 females was the coat color pattern (Figure 4). On first inspection there appeared to be patches of full-colored, light-colored and white fur. On closer inspection it could be seen that the colors of individual hairs within

a patch were variable. The distribution of the light and dark patches was characteristic and very different from that seen in animals carrying the various "spotting" genes, *s, W, bt, Va,* etc. In dappled animals patches having well-defined edges rarely crossed the mid-dorsal or mid-ventral line of the animal. This was particularly noticeable on the head, which was often divided by a line from ears to nose-tip into light and dark halves.

In addition all dappled females had curly vibrissae, although the degree of curliness was variable. In cases where the hair color was very different on the two sides of the snout, the vibrissae were more curly on the lighter side. In some animals the fur also was slightly wavy and of a finer texture than normal.

Another quite distinct abnormality of dappled animals was incoordination of the limbs. This was not seen in all dappled mice and when it occurred was first detectable as a tremor at a few days of age. Within the next few days the tremor became worse, the affected animals lost all coordination of the limbs and died at about two weeks of age.

Some dappleds a few months old or more showed yet another abnormality: calcified lumps either attached to the bones or free in muscles or tendons. These lumps were commonly seen attached to the vertebrae or the bones of the feet, but were also common in the limb musculature (Figure 5*B*). The frequency of these calcifications increased with the age of the animal.

As the spotting pattern of dappled-2 females was so different from that of all other spotted mutants of the mouse it was suspected that the cause of the spotting in dappled mice was different. Silvers[9] has shown that in the white fur regions of spotted animals the hair follicles lack pigment cells, whereas in albino animals the hair follicles contain cells resembling pigment cells but which lack pigment. Sections of skin of seven-day-old dappled females were examined. All hair follicles contained either pigment cells or cells which resembled pigment cells but lacked pigment (Figure 5*A*). Those follicles which contained pigment showed a range of variation from completely normal pigmentation to almost none. This was to be expected from the gross appearance of the coat.

Hemizygous Males

In an attempt to find the time of death of the postulated hemizygous dappled males, dappled females were killed and dissected when 11 to 14 days pregnant and their embryos examined; the results are shown in Table III. When females were opened at 13½ to 14½ days' gestation 16/54 embryos were dead and too decomposed for any abnormalities to be recognized. It was suspected that these represented the hemizygous males. Ac-

TABLE III. Embryo counts in dappled females pregnant by normal males

Age of embryos in days	No. uteri	Type of embryos: Normal	Dead embryos	Moles	Others	Total
14½	3	13	9	8		30
13½	3	14	7	2	1*	24
12½	3	15	Dead at 12 days: 4	3		22
11½	6	29	Blood in yolk sac: 5 + 1 dying	1	4†	39

*Subcutaneous hemorrhages present.
†2 dead at 9-10 days, 2 damaged.

cordingly another series of females were opened at earlier stages. At 12½ days recently-dead embryos were found; there were no visible external abnormalities but the embryos were white-looking and the yolk-sac circulations were empty. At 11½ days one embryo was moribund and five others were still living but had hemorrhages inside the yolk sac. It is accordingly postulated that these embryos that died between 11 and 12 days' gestation represented the hemizygous males. Considering all embryos examined at 12½ to 14½ days' gestation, these animals totalled 20/76 or 26.3 percent, in good agreement with the 25 percent which the hypothesis requires. The cause of death is not clear but may involve the circulatory system.

Comparison with Other Alleles

The only clear differences found so far between mottled alleles have been in the putative hemizygous males. Falconer[2] found that *Mo* males died at about 11 days' gestation with no visible external abnormality. Thus in this respect dappled-2 is indistinguishable from *Mo*. Dappled-2 females show some features not reported for *Mo*, such as incoordination of the limbs and calcareous deposits, but as only brief reports of the abnormalities of the affected females have been published for the other alleles this may not be a real difference. Dappled-2 is therefore considered to be indistinguishable from *Mo*.

Discussion

The mottled one is a locus or complex locus at which mutations occur fairly frequently. There are at least three different mutant alleles known, but as one of these has apparently been found at least twice (mottled and dappled-2) the number of possible alleles is probably not indefinitely large.

The distribution of the patches of hair of the various colors in mottled mice resembles that reported by Russell and Major[8] for the distribution of spots due to somatic mutation. Intermingling of hairs of the different colors also is seen in spotting due to somatic mutation. This suggests that the pattern of mottled mice is the result of the cells responsible for pigmentation becoming differentiated early in the cell lineage into those which will give rise to a colored hair and those which will give rise to a white hair.

In the case of the heterozygous females there could be some form of somatic segregation, with the mutant allele being effective in some cells and the normal allele in others. But the original mutant male showed the same pattern, and if he were of the normal XY chromosome type somatic segregation would be ruled out. It has recently been shown, however, that mice of the chromosome type XO are fertile females[10] and hence that the type XXY might be fertile males. It is thus possible that at least some of the cells of the original dappled-2 male may have been XXY. This is considered unlikely since, judged by his breeding behavior, his gonads were probably of a normal chromosomal type. An XXY male would be expected to form gametes of the types X, Y, XX and XY in unknown proportions, giving zygotes of types XX, XY, XXX and XXY. It is unknown whether XXX animals would live and therefore it is not possible to predict the phenotypes of such a male's female offspring, but his male offspring should include some XXY animals like himself. In the present case no dappled males were found and hence there is no evidence that the gonads of the original mutant male were XXY in type. It is postulated that he was a mosaic for a mutation which affected some cells of both the germ-line and the soma, and that his coat pattern was the result of mingling of the descendants of the mutant and non-mutant cells. This prompts the suggestion that in heterozygotes some event occurs at an early embryonic stage which determines whether the mutant or normal gene shall be active in each of the cells present at that time, and that the subsequent pattern depends on movement of the descendant cells. It is not possible to suggest what is the nature of this early event.

Summary

A new mutation at the sex-linked mottled locus of the house mouse was found in a male who was a gonosomic mosaic. The new allele is indistinguishable from the first known allele, *Mo*.

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A NEW MUTATION OF *a* TO *a*^{t} IN THE MOUSE

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IN MICE, mutations of genes at the agouti locus to a^t (black-and-tan) appear to be relatively common; six probable mutations to a^t are listed by Grüneberg[1]. The present report records a new occurrence of a^t resulting from the mutation of *a* (non-agouti) in a non-agouti polydactylous descent of mice.

The mutant mouse was observed in the seventeenth inbred generation following a cross of the polydactylous stock to C57BL. Its pelage was characterized by a white ventrum sharply demarcated on the flanks from a plush (black-brown) colored dorsum. A backcross demonstrated the condition to be inherited as a dominant and the mutant line has since been maintained by brother-sister matings. Comparison of mutant mice with a^t mice subsequently obtained from the Roscoe B. Jackson Memorial Laboratory revealed an identical pattern of light-belly except that the ventrum was white in the former and yellow-orange in the latter. However, earlier studies had shown that coat color in the Strong polydactylous stock of mice was complicated by the presence of chinchilla genes (c^{ch}) known to cause a marked reduction in yellow (phaeomelanic) and a slight reduction in black (eumelanic) hair pigments[2]. To test the validity of the impression that the new mutation was indeed black-and-tan modified by chinchilla genes at the *c* locus[3], mutant mice with a^t on a black (*B*) background C57BL/St (*aaBB*), BRS/St (*aabb*), C3H/St (*AABB*) and YBR/Wi/Ha ($A^y abb$) strains. The results are summarized in Table I.

The (mutant $\times$ C57BL)F_1 mice were essentially indistinguishable from mice with a^t on a black (*B*) background as would be expected if the genotypes of the hybrids were $a^t aBBC^{ch}$. In both cases the grey cast of the ventrum resulted from the darkly colored bases of the yellow-orange tipped hairs. The order of dominance for genes at the

(*Continued on page* 135)

TABLE I. Interaction of mutant "light-belly" gene with several genes of agouti series

F_1 of mutant x:	Agouti locus gene tested	Coat color	
		Dorsum	Ventrum
C57BL/St	*a*	Black	Yellow-orange with gray cast
BRS/St	*a*	Black	"
C3H/St	*A*	Bl.-agouti	"
YBR/Wi/Ha	A^y	Yellow	Yellow
	a	Black	Yellow-orange with gray cast

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AN INTERACTION PRODUCT OF THE CELLULAR ANTIGENS IN SPECIES HYBRIDS

Between *Gallus gallus* and *Phasianus colchicus*

M. M. Bacharach, W. H. McGibbon and M. R. Irwin*

ANTIGENIC specificities not possessed by either parental species have been noted on the red blood cells of various species hybrids in birds by Irwin[9], Irwin and Cole[10], McGibbon[13], Irwin and Cumley[12], Bryan and Miller[5], and Miller[15]. Cohen[6] has described an immune isoantibody in rabbits which presumably identifies an antigen resulting from the interaction of a pair of allelic genes. The new specificity, or "hybrid substance," of the cells represents an exception to the concept that the antigens of the red blood cells are more or less direct products of their causative genes[10, 7].

Serological studies of hybrid progeny between pheasants and domestic chickens were reported by Sasaki[17]. Using the precipitin technique, this author tried, without success, to demonstrate a specific protein peculiar to the generic hybrids. Inasmuch as the precipitin test is considered less discriminating than the agglutination test for the study of species relationships[2], a more sensitive method of testing the antisera might lead to different results.

The erythrocytes of the F_1 hybrids of the cross of the domestic fowl (*Gallus gallus*) and the ring-necked pheasant (*Phasianus colchicus*) have been found to possess a hybrid substance. The identification of the hybrid substance in the F_1 and its absence in related species and sub-species form the basis of this report. A description of hybrids between pheasants and chickens has been given by Shaklee and Knox[18].

Materials and Methods

The chickens used in this study were part of the flock maintained by the Department of Poultry Husbandry, while the pheasants were procured from the State Experimental Game and Fur Farm, Poynette, Wisconsin, through the courtesy of Mr. H. E. Stanz, Jr. Three F_1 chicken/pheasant hybrids were sired by a Rhode Island Red male (3151) in natural mating; two F_1 pheasant/chicken 21-day-old embryos were obtained by artificial insemination of S. C. White Leghorns with pheasant semen.

The hybrids derived from the cross between the chickens and pheasants were sterile, and therefore backcross progeny could not be obtained. Blood samples of two or three individuals in each of several related species were made available through the courtesy of Mr. W. A. Osburn, Manager of the State Experimental Game and Fur Farm. Blood samples of 20 Broad Breasted Bronze turkeys (*Meleagris gallopavo*) were furnished by Dr. M. L. Sunde of the Department of Poultry Husbandry.

The species whose red blood cells were examined are members of various genera of the family Phasianidae. The cells of representatives of the following genera and species were tested: *Alectoris graeca chukar*, the Chukar partridge; *Gallus gallus*, the domestic chicken; *Syrmaticus reevesi*, the Reeves' pheasant; *Phasianus colchicus colchicus*, the English Black-necked pheasant; *P. c. mongolicus*, the Mongolian pheasant; *P. c. torquatus*, the Chinese Ring-necked pheasant; *P. c. formosanus*, the Formosan Ring-necked

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pheasant; *P. c. mut. tenebrosus,* the melanistic mutant pheasant; *Chrysolophus pictus,* the golden pheasant; and *Chrysolophus amherstiae,* the Lady Amherst's pheasant.

Immunizations were made by intravenous injections of the pooled red blood cells of the hybrids into the sire of the F_1 chicken/pheasants, into pheasants, and into rabbits. The chicken and each of three pheasants received approximately 0.75 cc of a 50 percent cell suspension every third day, while 11 rabbits were injected with about one cc of a 10 percent suspension every other day. Naturally occurring agglutinins against the erythrocytes of the hybrids were not found to be present in the sera of the three pheasants nor in the sera of the 11 rabbits. However, the serum of the chicken sire of the hybrids (3151), as well as the sera of 70 chickens of various breeds, caused the erythrocytes of the hybrids and pheasants to be agglutinated.

The antisera obtained from the parental chicken and from the rabbits were processed and stored at -20 ± 5°C. until used. The pheasants were bled into test tubes containing a measured volume of sodium citrate solution (two percent sodium citrate and 0.42 percent sodium chloride). The plasma was probably reduced to serum during the incubation in a 56°C. waterbath for 30 minutes to destroy complement activity[8].

The pertinent immunological techniques, such as absorption of the antisera and agglutination tests, were essentially similar to those described by Irwin and Cole[10], Irwin *et al.*[11], and Briles *et al.*[4]. Briefly, testing fluids or reagents were prepared from pheasant antisera by absorbing one-cc portions of a particular antiserum diluted 1:8 with an equal volume of packed red blood cells of the sire (3151), pooled cells of pheasants, a combination of cells of pheasants and of the sire, or cells of the hybrids used to produce the antiserum. A detailed description of these techniques was given by Bacharach[1].

Experimental Results

The antisera produced either in the parental chicken (3151) or in the 11 rabbits did not contain antibodies toward a hybrid substance of the species hybrids. In the anti-F_1 serum from the chicken, naturally occurring antibodies to the erythrocytes of pheasants and the species hybrids masked the results. The absorption of the rabbit anti-F_1 sera with the cells of pheasants produced a reagent which agglutinated the cells of chickens, as well as the cells of the hybrids. Likewise, absorption of the rabbit anti-F_1 sera with the cells of chickens provided a reagent which reacted only with the cells of pheasants and hybrids. Thus, the erythrocytes of the hybrids presumably possessed most, if not all, of the antigenic specificities which are peculiar to chickens and pheasants, respectively, in contrast to each other (Table I).

Antisera to the cells of the hybrids, produced in pheasants, were strongly reactive (1:512) with the cells of chickens and the species hybrids, and only slightly reactive (1:2) with those of pheasants, as shown in Table I. When these antisera were absorbed with the cells of the sire (3151), the cells of the species hybrids and of five pheasants were agglutinated. However, reagents prepared by absorbing the anti-F_1 serum with the pooled cells of the sire (3151) and pheasants (*P. colchicus*), not related to the five mentioned above, caused the agglutination only of the cells of the species hybrids. Absorption with the cells of pheasants other than members of the species *P. colchicus* was not made. Since parallel results were obtained by the use of the antisera from each of three pheasants, only those obtained with antiserum 980P are listed in Table I.

The reagent specific for the hybrid substance did not agglutinate the erythrocytes of any of the pheasants belonging to the genera *Syrmaticus* or *Chrysolophus*. Likewise, none of the blood samples taken from partridges or turkeys showed reactions with this test fluid.

In tests with the reagent specific for the hybrid substance prepared in pheasant, the erythrocytes of five of 15 birds representing sub-species of *P. colchicus*

TABLE I. Results of testing the erythrocytes of several species of domesticated birds with anti-F_1 reagents prepared from chicken (3151), pheasant (*P. colchicus*) and rabbit antisera

Test cells	Anti-F_1 (chicken)	Anti-F_1 (pheasant, 980P)				Anti-F_1 (rabbit)				
	Absorbed by cells of pheasant	Titer unabsorbed	Absorbed by cells of: Chicken	Pheasant	Chicken plus pheasant	Titer unabsorbed	Absorbed by cells of: Chicken	Pheasant	Chicken plus pheasant	Species hybrid
Gallus gallus (3151)	0*	1:512	0	+	0	1:3840	0	+	0	0
Gallus gallus (A2003)			+†		+†					
F_1 chicken/pheasant hybrids	0	1:512	++	+	1:64	1:3840	+	+	0	0
F_1 pheasant/chicken hybrids (Cells from embryos)			++		+					
Phasianus colchicus	0	1:2	+	+	+	1:3840	++	0	0	0
	0	0	0	0	0	1:3840	++	0	0	0
Chrysolophus pictus			0		0		+++	0		
Chrysolophus amherstiae			0		0		+++	0		
Syrmaticus reevesi			0		0		+++	0		
Alectoris graeca			0		0		+++	+		
Meleagris gallopavo			0		0		+++	0		

*Symbols: 0 indicates lack of agglutination; + denotes agglutination. †Reactions before absorption with cells of A2003.

were agglutinated. These five comprised one English Black-necked pheasant, one Chinese Ring-necked pheasant, and three Formosan Ring-necked pheasants. Unfortunately, normal sera from the pheasants which served as sources of the antisera which contained the specificities of the hybrid substance were used up before the hybrid substance had been found. However, the normal sera of three pheasants related to those which served as sources of the anti-F_1 serum failed to react with the cells of these five pheasants, suggesting that immune antibodies were responsible for the agglutination of the cells of the hybrids.

Separate portions of the antiserum containing the specificities of the hybrid substance were absorbed with the cells of each of the five pheasants in question. In each case the erythrocytes used in the absorptions removed the reactivity for themselves, as well as for those of the other four birds, but not for those of the generic hybrids. Thus, the reacting specificity on the erythrocytes of these five pheasants probably represented individual differences among pheasants.

The erythrocytes of one of the chickens (A2003) likewise were agglutinated weakly (1:2) by the reagent prepared by absorbing antiserum 980P with the pooled cells of the sire (3151) and pheasant. The addition of the cells of A2003 to this combination of cells in absorption then resulted in a test fluid which reacted only with the erythrocytes of the species hybrids (1:64).

The agglutination of the cells of the hybrids from the reciprocal matings by this reagent, and the lack of agglutination of the cells of the parental species are evidence for the presence of a new specificity, or "hybrid substance," on the cells of the F_1 hybrids. The presence of the hybrid substance complex on the erythrocytes of only the species hybrids when contrasted to its absence on the red blood cells of the parental and related species lends support to the belief that the hybrid substance is the result of complementary action of genes from each parent, which by virtue of their interaction in the species hybrid produce a

different specificity than either can produce alone.

Discussion

The hybrid substances detected so far are probably due to the interaction of genes from their respective parental species. Except for the data on rabbits[6], evidence for hybrid substances has been found only in species and generic crosses. It appears reasonable to predict the future isolation and characterization of additional hybrid substances in rabbits and chickens, as well as in other species, where breeding records of large populations have been kept. However, it should not be anticipated that the antigenic configurations of these interaction products will be identified readily.

The total sterility of the species hybrids precluded any offspring from either *inter se* or backcross matings. Thus, it could not be determined whether the genes presumably responsible for the hybrid substance were located on sister chromosomes or on independent chromosomes. There is insufficient information available on the antigenic constitution of pheasants and chickens to offer any clues on this point at the present time. Miller and Bryan[16] and Bryan and Miller[5] adduced evidence that a hybrid substance in the blood cells of progeny from matings between *Columba guinea* and *C. livia* was presumably due to the interaction of genes, possibly but not certainly alleles, on a pair of contrasting chromosomes.

It is significant that the antigens of the hybrid substance were found to be expressed to the same degree in the two 21-day-old embryos as in the adult species hybrids. This seems to indicate that the hybrid antigens are formed during the developmental period of the embryo in a manner similar to the species-specific ones. Briles *et al.*[3] obtained agglutination of chick embryo cells of appropriate genotypes with their "B" reagents as early as the third day of incubation. Miller[14] found certain species-specific antigens of *Columba guinea* present in backcross hybrids with *C. livia* by 72 hours of incubation.

The agglutination of the erythrocytes of the five pheasants, all subspecies of *Phasianus colchicus*, by the reagent for the hybrid substance from antisera of pheasants prior to the absorption with the cells of the reacting individuals points to a high degree of complexity of the antibodies characterizing the hybrid substance. Since the cells were agglutinated by the anti-F_1 reagent, but not by the normal pheasant sera, the antiserum may have contained agglutinins which cross-reacted with the erythrocytes of the five pheasants. A second, but less likely, explanation for these reactions would be to postulate the presence of naturally occurring antibodies in the anti-F_1 reagent which agglutinated the erythrocytes of the five pheasants.

The possibility cannot be excluded at this time that the reaction of the cells of the five pheasants with the pheasant anti-F_1 reagent was due to the action of two different loci on two different chromosomes in the pheasant species; that is, nonallelic interaction had occurred. Furthermore, it is possible that pheasants maintain a state of perpetual heterozygosity for the genes which interact to produce the hybrid substance by possessing a system of balanced lethals. Whether or not the reactions of the erythrocytes of these five pheasants were the result of the same agglutinins which caused the agglutination of the cells of A2003 (the same reagent being involved) could not be determined, since A2003 was no longer available when the erythrocytes of the various species were tested.

The data dealing with species relationships have been summarized in Table I. The presence of the hybrid substance specificity on the erythrocytes of the species hybrids, in contrast to its absence from the cells of the species represented, may be taken as evidence favoring the hypothesis that the hybrid substance very likely expresses the product of complementary genes from *P. colchicus* and *G. gallus*, which by virtue of their interaction in the species hybrid produce a different specificity than either can produce alone.

Considerable difficulties were experienced in producing and rearing the species hybrids used in this study. Apparently, the differences in chromosome complements of the parental species provided the hybrids with selective disadvantages in development and growth. It appears extremely unlikely that successful hybridization between members of the genus *Phasianus* and the genus *Gallus* have occurred in the past in nature which could have resulted in the creation of new species.

Summary

The red blood cells of chickens, pheasants, and hybrids between these two species have been examined for antigenic differences by means of immunological techniques. The erythrocytes of the F_1 hybrids were shown to possess most of the antigens specific to the parental species and probably all those held in common by both. In addition, the cells of the hybrids were shown to possess a new specificity, or "hybrid substance," which was not present in the blood cells of either parent. A few representative birds of related species were studied for the presence of the hybrid substance specificity. The absence of this specificity from the erythrocytes of the related species, when contrasted to its presence only in the species hybrids, suggests that the hybrid substance is the result of complementary action of genes from each parent, which by virtue of their interaction in the species hybrid produce a different specificity than either can produce alone.

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CORRIGENDUM

An error appeared in the first subheading of Table II, page 38 of the article by Casady *et al.*, "Inheritance of Female Sterility in Sorghum," which appeared in the January-February 1960 issue of the *Journal*. The subheading as it should read is as follows:

Westland × [Early Kalo, 231 × *fs* (Westland × Early Kalo, 231, F_1), BC_1]

The editor regrets the error.

GENETICS OF THE "BROWN" HORSE

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UP to the present time most mammalian geneticists, including the senior author, have used the term *brown* to designate only the genotype which is homozygous for the recessive *b* allele of the gene for black pigment, *B*. In horses, this would restrict the term to genotypes of chestnut and sorrel, and their subordinate classes, such as palomino and cremello.

But other students of horse genetics, in particular Salisbury[6] and Odriozola[4], recognize among basic color groups, which include both bay and black, a third black-pigmented group intermediate between bay and black and popularly known as brown, black-brown, brown-black, or seal brown. The present article is concerned with the genetic constitution of this intermediate group.

Bay and black genotypes differ only as regards the ancestral wild-pattern gene, *A*. This pattern is present in bay and absent in black, the respective alleles involved being *A* and *a*. It would be logical to suppose that a type intermediate between bay and black would involve a different allele of *A*. This Odriozola alone has had the perspicacity to recognize and has designated the third allele a' after analogy with the black-and-tan rabbit, which occupies a similar intermediate position between agouti (*A*) and black (*a*), as was clearly demonstrated by Punnett[5], who designated the black-and-tan allele a'.

The agouti (wild-type) rabbit has black hair, with barred tips on its back and sides, with a whitish belly where the hairs lack the barred tip. The black rabbit lacks altogether the regional color differences found in the gray rabbit, its entire coat being black, only less heavily pigmented ventrally.

The black-and-tan rabbit has a reduced and modified expression of the agouti pattern. Its back and sides are entirely black except for some guard hairs with agouti tips far down on the sides near the whitish belly, where the barred tips are wanting, as in the gray rabbit.

In horses the group intermediate between bay and black has a reduced and modified expression of the bay pattern.

Salisbury describes the three color classes, bay, brown and black, as follows:

1. *Bay.* A bay horse or pony is one having a black mane and tail, black feet and legs, and black often extending as high as the knees and hocks, and a red-colored body of several different shades, which may vary from a light bay through the various intermediate shades to a dark cherry red.

2. *Brown, brown-black* or *seal brown.* The brown color is sometimes confused with bay and, if the individual is especially dark, may be confused with black. It is accompanied by black mane, tail, feet, and legs. The body is brown varying to black, but the muzzle, the region about the eyes, the flanks and underlines, especially in the groins, are a lighter shade of brown. These "light points" are variable in extent and intensity of pigmentation, which fact has led to confusion in the identification of animals for registration purposes.

3. *Black.* Black is a uniform color found most commonly in Percheron horses and Shetland ponies. It is particularly subject to bleaching in sunlight. Bleached black horses exhibit a marked red tinge to the hair which approaches bay or brown in color.

Salisbury also states that "horses which later are black are usually a peculiar mouse-brownish-gray color at birth. This disappears with the foal hair. If black horses are subjected to intense

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THE "BROWN" HORSE

Figure 6

Animals of this type, sometimes confused with bay or black depending on the extent and intensity of the pigmentation of their "light points," are actually members of a distinct and genuinely basic color class. The brown phenotype is conditioned by an allele of the bay gene, a^t. It interacts with the B and E genes and their alleles to produce black or brown coat color.

sunlight, the color may be bleached to a reddish tinge which is not far different from bay. Occasionally an individual may be jet black when foaled, with the color changing little, if any, when the foal hair is shed. This however is the exception."

We regard these exceptional non-fading jet blacks as dominant blacks, possessing a special allele of the extension gene E, viz. E^D, which allele so increases the amount of black pigment in the coat as to hide the presence of an A allele.

Salisbury, unlike Odriozola, has not named any gene or genes concerned in differentiating brown from bay and black, but has merely tabulated certain stud-book records for the results of matings within or between the three groups.

The data assembled by Salisbury from records for Shetland ponies are reproduced in Table I, omitting the relatively small number of matings in which the colt was a chestnut or sorrel and in

which both parents must have been *Bb* in genotype.

The data in Table I are in harmony with the assumption that the order of dominance among *A* alleles is the same in horses as in rabbits, viz. *A*, a^t, *a*, but the occurrence of unexpected classes in certain matings requires further consideration.

The description given by Odriozola of the brown, black-brown, or seal brown horse (*bocifuego* to him) agrees well with that given by Salisbury, but is even more detailed. He states that the general body color is black but becomes lighter by a change to a reddish yellow color in the muzzle, around the eyes, on the ears, the neck, the flank, and the anal region.

Neither Salisbury nor Odriozola name any specific genetic agency to explain the variation in the intensity or extent of the light areas in the color patterns of bay and brown horses. But we are able to name one such agency overlooked by them, viz. the gene *E* for extended or restricted distribution of black (or brown) pigment in the coat, an agency well known to students of mammalian color genetics.

TABLE I. Results of matings between or within color classes bay, brown, and black in Shetland ponies.

Parents	Colts			
	Bay	Brown	Black	Total
Bay × Bay	375	65	71	511
Bay × Brown	346	188	126	660
Bay × Black	929	428	921	2,278
Brown × Brown	51	133	81	365
Brown × Black	304	737	1,222	2,263
Black × Black	251	407	4,406	5,064
				11,041

TABLE II. Genotypes and expected phenotypes, if *E* alleles are included in the genetic formulae

Genotype	*Phenotype*
ABE^D	Jet black, dominant black
ABE	Mealy or dark bay, body color sooty red or yellow
ABee	Light bay, body color clear red
a^tBE^D	Jet black, dominant black
a^tBE	Black-brown, light areas inconspicuous
a^tBee	Black-brown, light areas conspicuous
$aaBE^D$	Jet black, dominant black
aaBE	Uniform black, less intense than foregoing and fading; recessive black
aaBee	Black with mane and tail darker than body; sometimes registered as black with black mane and tail.

This gene has three recognized alleles: 1) E^D, dominant extension, well illustrated in the dominant black rabbit, as shown by Punnett; 2) *E*, ordinary full extension of black throughout the coat as in the black rabbit; 3) *e*, restricted distribution of black pigment in the coat, chiefly to the extremities, leaving the body red or yellow in color, as in the yellow or fawn rabbit.

If we include the *E* alleles in the genotypes of bay, brown, and black horses, the resultant phenotypes to be expected are as shown in Table II.

If now we examine Table I in the light of Table II, we can better understand the surprisingly large size of the classes expected to be small or nonexistent in particular matings.

Since bays may be heterozygous for alleles a^t or *a*, it is not surprising that bay × bay matings should produce considerable numbers of brown and black colts, and that these numbers should be still larger in matings bay × brown and bay × black.

Brown × brown matings are expected to produce some black colts, if the parents are of genotype a^ta, but they are not expected to produce any bays, though they do according to the records. This might well be because the colts were of genotype a^tBee, and in which the light areas were so conspicuous as to make the colt resemble bay and be so reported.

Black × black matings are expected to produce only black colts. The occasional production of a brown colt might result if one parent, nominally black, was really a brown of genotype a^tBE, in which the body pattern was so inconspicuous as to be overlooked. A bay colt might result occasionally from a black × black mating if one parent was a jet black of genotype ABE^DE (or *e*).

Summary

The color group in horses known as brown, black-brown, or seal brown is a distinct and genuinely basic color class, like bay and black, between which it occupies an intermediate position. It is differentiated from them by a different allele of the ancestral coat-pattern gene, *A*. The respective alleles and their order of dominance are *A* bay, a^1 brown, *a* black. Brown in horses has the same relation to bay and black as has black-and-tan to gray and to black genotypes in the rabbit. For this reason the same genetic symbol is used in both cases, as suggested by Odriozola.

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THE BLACK MUTATION IN TRIBOLIUM CASTANEUM

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FOR many years, two species of beetles, *Tribolium castaneum* Herbst and *T. confusum* Duval (Tenebrionidae), have been investigated from the standpoint of population ecology. In both species, spontaneous mutations have appeared from time to time in laboratory cultures: in *T. confusum*, Park *et al.*[5] reported ebony, a recessive mutation, and Stanley and Slatis[8] a semi-dominant gene, McGill black. Both of these mutations affect body color. Viewed with the naked eye both forms appear black. However, if the beetles are viewed under the microscope, ebony beetles are purplish in color, and McGill black beetles are also purplish, but darker. The McGill black heterozygote (called bronze) is closer to the wild type (dark red or chestnut), but it is easily distinguished from the latter. McDonald[1] has discovered two additional mutations: split, an autosomal recessive, which shortens the elytra exposing the posterior third of the abdomen, and striped, a sex-linked lethal mutation, which modifies the pigmentation of the elytra.

In *T. castaneum* three mutations have been described. Pearl, an autosomal recessive gene described by Park[3], affects the pigmentation of the eye. Paddle, a recessive sex-linked gene reported by Park and Frank[7], causes a variable fusion of the three broad terminal antennal segments in the female. In the male, in addition to a more pronounced fusion of the three terminal segments, there is a reduction in the number of antennal annuli. The third, a semi-dominant gene affecting body color, has been reported unchristened by Miller[2]: the heterozygote is darker than, and distinguishable from, the normal, or homozygous wild type. The mutant homozygote appears black.

Two similar mutations, probably allelic to that described by Miller†, have recurred spontaneously in two wild-type strains of *T. castaneum* at about the same time. One wild-type strain, identified as McGill red, has been maintained by one of us (J. S.) for some 30 years. The other wild-type strain (hereafter to be referred to as Chicago red) has been maintained by Dr. Thomas Park, University of Chicago, for about the same length of time.

As in *T. confusum* McGill black, no differences can be observed at the egg stage between *T. castaneum* wild type and the mutant. However, larvae as small as 1.5 mm can be distinguished readily with a 40× magnification. In wild-type larvae, the head, tergites and cerci are reddish; in the homozygous mutant, the head and tergites appear greyish but the cerci are darker, tending to be black at the tip.

With some training, one can distinguish heterozygous from wild-type young larvae by examining the broad part of

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†Since Miller's mutation is no longer in existence, it was not possible to carry out tests of allelism with this form.

the cerci; the cerci in the heterozygote are dark red, while in the wild type the cerci are a paler red.

Large larvae can be classified into homozygotes and heterozygotes at low magnification (13×) by the appearance of the cerci, tergites or head, and, in addition, by the color of bristles, jaws and tarsi.

Freshly formed pupae cannot be classified as to genotype. Furthermore, since the color of the heterozygote is very close to the wild type, and the pupal cerci are very narrow, the pupae can be classified with certainty into only two groups, namely, pupae which will become black beetles and pupae which will become heterozygotes and homozygous wild type.

Freshly hatched beetles are unpigmented, and must be aged (preferably for about a week) before classification is possible. At this time, all sclerotized parts (including the penis) acquire the pigmentation characteristic of the particular genotype. Under the microscope the homozygous mutant is purplish black, the heterozygote a dark red which will be referred to as "bronze," and the normal is red. While classification into the three phenotypes is possible with the naked eye, it is more reliable (and less tiring) to use the low power of the dissecting scope to determine the phenotype of the beetles. With the aid of the microscope, bronze and red beetles can be classified into their corresponding phenotypes without error.

Because the bronze beetles are much closer to the red beetles in color than they are to the black, and because the caudal cerci of the bronze pupae are indistinguishable from those of red pupae, black will be given the small letter and the wild type the capital letter. There-

TABLE I. Progeny from various crosses of McGill red with McGill black

No.	Genetic mating	No. of crosses	Color of progeny: Red ♂♂	Red ♀♀	Bronze ♂♂	Bronze ♀♀	Black ♂♂	Black ♀♀
I.	B/B♂ × b/b♀	2	0	0	211	232	0	0
II.	B/B♀ × b/b♂	2	0	0	250	240	0	0
	$B/b \times B/b$							
III.	from I	5	23	28	38	44	13	14
IV.	from II	6	34	42	72	77	30	28
V.	B/B♂ × B/b♀	7	156	142	128	144	0	0
VI.	B/B♀ × B/b♂	7	116	111	115	106	0	0
VII.	B/b♂ × b/b♀	8	0	0	180	170	150	181
VIII.	B/b♀ × b/b♂	8	0	0	168	179	172	168

TABLE II. Progeny from various crosses of Chicago red with Chicago black

No.	Genetic mating	No. of crosses	Color of progeny: Red ♂♂	Red ♀♀	Bronze ♂♂	Bronze ♀♀	Black ♂♂	Black ♀♀
I.	B/B♂ × b/b♀	19	0	0	612	556	0	0
II.	B/B♀ × b/b♂	19	0	0	569	635	0	0
	$B/b \times B/b$							
III.	from I	19	142	159	291	318	144	158
IV.	from II	19	161	144	294	309	127	160
V.	B/B♂ × B/b♀	16	167	183	168	209	0	0
VI.	B/B♀ × B/b♂	19	207	212	180	188	0	0
VII.	B/b♂ × b/b♀	19	0	0	209	213	169	229
VIII.	B/b♀ × b/b♂	18	0	0	234	292	227	238

fore, black beetles may be designated as *bb*, bronze as *Bb*, and red as *BB*.

Results

Genetic tests have been performed in all possible combinations between the McGill strains and the Chicago strains. The results are summarized in Tables I through V.

The data are generally consistent with the hypothesis that black is a semidominant mutation. As can be readily seen (Tables I and II), matings of red × black in every possible mating combination within the McGill and the Chicago strains result in an F_1 which is bronze in appearance.

When the F_1 heterozygotes are mated, the resulting F_2 progeny (with one exception) do not deviate significantly from the theoretical ratio of 1 red (wild type) : 2 bronze (heterozygotes) : 1 black (homozygous recessive). The exception involves the progeny resulting from mating the McGill black male × McGill red female F_1's. The expected number of each phenotype when 160 individuals are produced is 40 red: 80 bronze: 40 black. The actual number was 51 red: 82 bronze: 27 black. This gives a χ^2 of 7.29 which for two degrees of freedom gives P = .02-.05, a probably significant deviation. Stanley and Slatis[8] observed a similar deficiency of black beetles in *T. confusum*, indicating a somewhat lower viability of the homozygous recessives. This lower viability of black beetles is not apparent in the F_2 produced by mating the F_1's of the reciprocal cross (i.e., McGill black female × McGill red male). Within the Chicago and McGill strains the backcross of the bronze heterozygote with black yields almost equal numbers of bronze and black beetles as expected. Mating of the bronze and wild type produces bronze and red beetles in almost equal numbers.

Generally, there is no effect on the expected ratios by introducing black into a different genetic background, i.e., by mating the Chicago black mutant with McGill red beetles and vice versa (Tables III and IV). In some instances, a deficiency of a certain phenotype, greater than that allowed by theory, is observed: in backcrossing F_1 males (of the cross

TABLE III. Progeny from various crosses of Chicago black with McGill red

No.	Genetic mating	No. of crosses	Color of progeny: Red ♂♂	Red ♀♀	Bronze ♂♂	Bronze ♀♀	Black ♂♂	Black ♀♀
I.	*B/B*♂ × *b/b*♀	2	0	0	212	234	0	0
II.	*B/B*♀ × *b/b*♂	2	0	0	194	187	0	0
	B/b × *B/b*							
III.	from I	6	52	48	93	94	46	44
IV.	from II	6	77	84	165	181	76	99
V.	*B/B*♂ × *B/b*♀	8	169	191	166	169	0	0
VI.	*B/B*♀ × *B/b*♂	6	131	121	96	102	0	0
VII.	*B/b*♂ × *b/b*♀	7	0	0	99	91	79	80
VIII.	*B/b*♀ × *b/b*♂	8	0	0	125	142	130	121

TABLE IV. Progeny from various crosses of Chicago red with McGill black

No.	Genetic mating	No. of crosses	Color of progeny: Red ♂♂	Red ♀♀	Bronze ♂♂	Bronze ♀♀	Black ♂♂	Black ♀♀
I.	*B/B*♂ × *b/b*♀	2	0	0	223	200	0	0
II.	*B/B*♀ × *b/b*♂	2	0	0	276	260	0	0
	B/b × *B/b*							
III.	from I	6	79	79	133	160	65	67
IV.	from II	5	50	63	157	137	62	77
V.	*B/B*♂ × *B/b*♀	7	146	154	167	143	0	0
VI.	*B/B*♀ × *B/b*♂	7	142	177	160	158	0	0
VII.	*B/b*♂ × *b/b*♀	6	0	0	140	125	141	138
VIII.	*B/b*♀ × *b/b*♂	6	0	0	132	132	133	107

Chicago black male and McGill red female) to the wild type (red), one cross gave 60 red and 39 bronze beetles ($\chi^2 = 4.46$, df = 1, P = .02-.05).

In the reciprocal cross between these two strains (Chicago black female × McGill red male), in one case (in which the F_1 male was backcrossed to the Chicago black female) and in another (in which the F_1 female was backcrossed to a Chicago black male) again there was a deficiency of blacks. In the first culture there were 53 beetles of which 34 were red and 19 bronze ($\chi^2 = 4.24$, df = 1, P = .02-.05). In the other culture there were 67 beetles of which 48 were red and 19 bronze ($\chi^2 = 12.58$, df = 1, P = <.01).

In a backcross to wild type there was a deficiency of bronze beetles. Of 54 beetles examined 35 were red and 19 bronze ($\chi^2 = 4.74$, df = 1, P = .02-.05).

These few instances are so rare (in comparison to the total number of replications not deviating from the expected) that one must attribute the finding of such cases as due to chance alone, since the over-all data do not deviate significantly from the theoretical ratios.

Matings of Chicago black with McGill black in all possible mating combinations have been carried out for two generations (Table V). Only black beetles were produced, indicating that these mutations are one and the same.

Several conclusions can be made from the genetic data: 1) black is a semidominant mutation; 2) except for chance effects there does not appear to be a differential viability between the different genotypes up to the stage at which classification to phenotype is made; and 3) evidence from reciprocal crosses shows that this gene is not linked with sex, but is autosomal.

In dioecious organisms having the XY method of sex determination, equal numbers of males and females should be observed among the progeny. Smith[9, 10, 11] has shown that both *T. castaneum* and *T. confusum*, although differing in the number of pairs of autosomes, have the XY method of determining sex.

When the sex ratio is considered, there are a few instances (particularly involving the Chicago strains) in which there is a significant deviation from the expected ratio of 1 male: 1 female. These are summarized below:

Mating	Progeny ♂♂	Progeny ♀♀	χ^2	df	P
McGill black ♂ × McGill red ♀	25	42	4.32	1	.02-.05
McGill black ♀ × McGill red ♂	41	23	5.06	1	.01-.02
McGill black ♀ × Chicago black ♂ F_1	96	133	5.98	1	.01-.02
McGill black ♀ × Chicago black ♂ F_2	66	44	4.40	1	.02-.05
Chicago red ♂ × Chicago black ♀	18	34	4.92	1	.02-.05
Chicago black ♂ × Chicago bronze ♀	16	35	5.66	1	.01-.02
Chicago black ♀ × Chicago bronze ♂	8	20	5.12	1	.02-.05

Because of the much larger number of replications, the Brandt-Snedecor test for homogeneity has been applied to each series of matings within the Chicago strains. The test fails to show any significant deviation from one vial to the next, so that we must regard the data of each set of vials as fairly homogeneous. Nevertheless, individual χ^2 tests for sex-ratio point to the above replicates as not falling in line with the expected ratios.

One could attribute these discrepancies in sex-ratio to chance alone, except for the fact that Park[3] and Park *et al.*[6] have observed a similar situation in other genetic work with *Tribolium*.

It is evident that the deviations from sex-ratio are a problem apart from the genetic ratios obtained for the semidominant gene black. If the factors responsible for modifications of sex-ratio are examined, they fall into

TABLE VI. Summary of sex ratio in the various crosses

	♂♂	♀♀
a) McGill red × McGill black	1856	1884
b) Chicago red × McGill black	2198	2179
c) Chicago black × McGill red	1910	1988
d) Chicago red × Chicago black	3901	4203
e) McGill black × Chicago black	886	962

TABLE V. Progeny from various crosses of Chicago black (1) with McGill black (2)

No.	Mating	No. of crosses	Color of progeny: Red ♂♂	Red ♀♀	Bronze ♂♂	Bronze ♀♀	Black ♂♂	Black ♀♀
I.	(1) ♂ × (2) ♀	2	0	0	0	0	154	205
II.	(1) ♀ × (2) ♂	2	0	0	0	0	163	179
III.	F_1 × F_1 (from I)	6	0	0	0	0	216	208
IV.	F_1 × F_1 (from II)	6	0	0	0	0	353	370

three categories: environmental, cytoplasmic and genetic. In this investigation there is no evidence that the first two factors are involved. It is possible that genetic factors, especially genes affecting sex-ratio or sex-linked lethals, may be in operation, but until more genetic markers are developed, these deviations in sex-ratio must remain unexplained.

Summary

Evidence is presented that the spontaneous mutations McGill black and Chicago black in *T. castaneum* are allelic and due to a semi-dominant autosomal gene. The homozygous mutant is black to the naked eye, but purplish-black if inspected with a microscope. The heterozygote is bronze (dark red) and close to, but distinct from, the normal or wild type.

The larval phenotypes can be distinguished readily at an early stage by examining the color of the cerci and the tergites. The pupae can be classified with certainty only to two phenotypes (black and non-black) because the cerci are too narrow to distinguish the two shades of red. Imagoes aged for a week can be classified to their corresponding phenotypes with little or no error. Where a genetic marker is needed in ecological work, the mutation black is the best available so far.

The action of the gene black is not affected by changing its genetic background.

Significant deviations in sex-ratio have been observed once again in the Chicago strain of *T. castaneum*. Because of the lack of adequate genetic markers, no explanation can be offered for these deviations at the present time.

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◆

A New Mutation of *a* to a^t in the Mouse

(*Continued from page* 121)

agouti locus included in this study was shown to be $A^y > A > a = a^t$ for dorsal color and $A^y > a^t > A > a$ for ventral color[1]. Thus the color types obtained in all outcrosses (Table I) were consistent with the view that the new mutation was a^t. Segregation of color types in the F_2 and backcross generations of all matings supported this interpretation.

Although remote ancestors of the polydactylous stock were treated with methylcholanthrene, the long period since such treatment (at least five years) suggests that the newly observed mutation of *a* to a^t occurred spontaneously.

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BLOOD TYPES OF THE PROGENY OF A PAIR OF CATTLE TWINS SHOWING ERYTHROCYTE MOSAICISM

W. H. Stone, D. T. Berman, W. J. Tyler and M. R. Irwin*

A HIGH proportion of dizygotic twins and calves resulting from multiple births in cattle has been shown by Owen[13] and others[14, 17, 18] to involve an intermixture of antigenically distinct kinds of blood cells derived from genetically different individuals. The terms "erythrocyte mosaicism"[14] and "chimerism"[1] have been used to describe this condition. The only reports of the appearance of this phenomenon of blood admixture between twins in species other than cattle are three cases in man[4, 6, 12] and one in sheep[19]. It would be surprising indeed if a parallel phenomenon did not occur in species other than these. (Erythrocyte mosaicism in man, presumably as a result of somatic mutation, has been reported[5] in individuals who were not twins.) Owen[13] proposed that the explanation of the mechanism operating in twins in cattle to produce the admixture of blood was provided by the previous report of Lillie[9]. Lillie had demonstrated the union of the circulatory systems of twin bovine embryos of opposite sex to explain, on an endocrine basis, the sexually modified female, known as the *freemartin*. Owen stated that the vascular anastomoses of the embryonic tissues provided a bridge across which the embryonal cells ancestral to the tissues forming the erythrocytes could migrate reciprocally from one twin to the other. The foreign cells become established in the hematopoietic tissues of the co-twin host and provide a source of blood cells genetically different from those of the host throughout life.

The two kinds of blood cells in the circulation of each twin can be readily separated by "differential hemolysis"[14], and an estimate made of the proportions in each twin of the two kinds of blood. (An improved spectrophotometric technique for measuring with ease and high accuracy the two kinds of blood cells will be described elsewhere[10].) However, it is not possible to determine which cell type belongs to the host (germinal) and which to the co-twin (somatic) except by data on the progeny of either or both individuals.

Owen[13] stated that a twin sire had failed to transmit to any of 20 progeny certain of the antigenic factors of his blood, implying that these antigenic factors were present somatically but not germinally. Stone and Palm[16] reported a disputed parentage case in bovine twins involving erythrocyte mosaicism. Two calves of a cow—one of a pair of twins—possessed certain blood groups which could not have been transmitted by the sire and which were expressed very weakly or not at all in the blood of the

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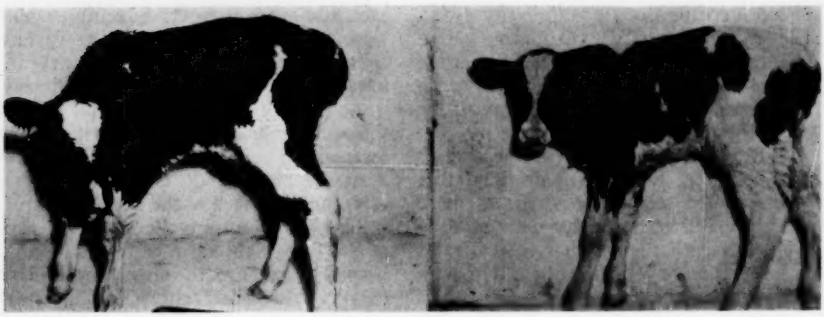

DIZYGOTIC TWINS

Figure 7

Obvious morphological differences confirm the dizygosity of these twins possessing erythrocyte mosaicism.

dam. The dam's blood was separated into two types: one constituting approximately 20 percent of the cell volume containing the previously unexplained blood groups of her calves and, therefore, representing the dam's own genotype; the other constituting approximately 80 percent of the cell volume, undoubtedly representing the genotype of the co-twin.

The present report describes the results of blood typing tests on the progeny of twin bulls with erythrocyte mosaicism. One purpose of these tests was to attempt to determine whether there were any effects of the intimate union, beginning in early embryonic life, of the two genetically distinct hematopoietic tissues on the germinal and somatic tissues of either twin.

Materials and Methods

The two male twins used in this study were of the Holstein-Friesian breed and were born into the experimental herd at the Emmons Blaine Jr. Farm of the University of Wisconsin in January, 1954. Blood samples were obtained from these twins at one week of age and blood typed using the hemolytic technique as described previously[20]. The results of this first blood typing test as well as of others over a period of four years showed that the twins possessed erythrocyte mosaicism and therefore were dizygotic. The prediction as to dizygosity was confirmed by their morphological differences which were readily apparent between the twins (Figure 7).

At eight months of age one twin (362C) was moved to another herd. The other twin (362B) remained in the herd at the Blaine Farm and was used in artificial breeding. Breeding began for both twins at about 15 months of age. Blood samples were obtained for blood typing from all of their calves when they were over one month of age. More than half of the calves were bled again at various ages and their blood retested.

Results

Erythrocyte mosaicism

The blood types of the twin bulls and their parents are shown in Table I. It can be seen that the twins possessed identical blood types. Two characteristic features of the blood types suggested that mosaicism existed. These were (1) the weak reactions (partial hemolysis) for several of the antigenic factors in each

TABLE I. Blood types of twin bulls and their parents

	GENETIC SYSTEMS ♂						
	B	C	F	J	L	Z	H^1
SIRE	$BGKO_3A^1/QE_1^{1,2}$	C_1X_1/C_1X_3	F/F	-/-	-/-	Z/-	H/-
DAM	$BGKO_3A^1/B_1$	$C_1X_3/?$	F/F	J/-	L/-	-/-	H/-
TWIN 362B	$QE^{1,2}/BGKO_3A^1/B_1$	C_1X_1/C_1X_3	F/F	J/-	±/-	Z/-	H/?
TWIN 362C	$QE^{1,2}/BGKO_3A^1/B_1$	C_1X_1/C_1X_3	F/F	J/-	±/-	±/-	H/?

[1]Representing only seven of the 10 or 11 known genetic systems.

[2]The diagonal line (/) separates alternative blood groups of a particular system. The dash (—) represents the absence of a reaction with any of the known typing fluids (reagents). The question mark (?) indicates that the alternative group is unknown. The plus-minus (±) indicates a weak reaction—partial hemolysis.

of five genetic blood group systems and (2) the necessity of postulating three phenogroups in the B system of each animal because any two known alternative phenogroups would not account for all of the reactions noted.

Differential hemolysis tests on samples of blood from each twin confirmed that mosaicism existed. The results of these tests are shown in Table II. Cells of Type I were not lysed after treatment of a suspension of cells from each twin with reagents for the antigenic factors I_1, L, Z, or H′. Cells of Type II were not lysed after treatment of the cells with reagents for G, K, O_3, A′ and X_1. The proportion of each cell type in each twin as determined by the microscopic cell counting technique as well as by the new spectrophotometric technique[10] was approximately 50:50. In other words, each twin possessed in approximately equal proportions the two antigenically distinct kinds of cells, Type I and Type II. Each of the two cell types in each twin, separable by differential hemolysis, was genetically compatible with those of the parents.

At least five differential hemolysis tests and numerous blood typing determinations were performed on the blood of these twin bulls at various intervals during the period of four years and the results were essentially parallel, showing that the proportions and kinds of the two cell types remained constant in each twin. Further, there was no demonstrable effect on either type of cell resulting from its association with cells of the other type.

Blood types of progeny

Each of the seven progeny of twin 362B and each of the 19 progeny of twin 362C was blood typed after it was one month of age. The reactions with the blood typing reagents of the cells from each of these 26 progeny were typical in each and every respect. Several progeny were blood typed repeatedly at various ages and the results obtained at each test paralleled those obtained previously.

As may be seen in Table III, each of the seven calves resulting from testcross matings of twin sire 362B *failed* to inherit the phenogroup BI_1 of the B system. Further, each of the five calves from testcross matings involving L and each of the five calves from testcross matings involving Z *failed* to inherit these blood groups from their sire. Similarly, the 19 calves of twin sire 362C *failed* to inherit the phenogroups $BGKO_3A'$ and C_1X_1 of the B and C systems, respectively. Since the inheritance of the blood groups of one genetic system is independent[11] of the inheritance of the blood groups of another system, we have considered that a single mating of 362B represents a testcross for one or more of the three systems B, L, and Z. In like manner, each of the 19 matings of 362C represents a testcross for both the B and C systems (or a total of 38 testcrosses).

It is apparent from these results that the groups BI_1, L and Z in the blood of 362B and the groups $BGKO_3A'$ and C_1X_1 in the blood of 362C are the "acquired characters" and are present somatically but not germinally. If one postulates that each twin is genetically heterozygous for each of these independent blood groups, the data can be regarded as contradicting a null hypothesis that each of the acquired characters (BI_1, L and Z of 362B and $BGKO_3A'$ and C_1X_1 of 362C) is transmitted with a probability of ½. Thus, $(\frac{1}{2})^n$, where n is the number of non-appearances of an independent blood group, gives the probability of the existence of the blood groups as heritable characters. Converse-

TABLE II. Blood types of the two kinds of cells in each twin bull

CELL TYPES	BELONGING TO TWIN ↓	GENETIC SYSTEMS						
		B	C	F	J	L	Z	H[1]
TYPE I	362B	$QE_1^1//BGKO_3A'$	C_1X_1/C_1X_3	F/F	J/-	-/-	-/-	-/-
TYPE II	362C	$QE_1^1//BI_1$	C_1X_3/C_1X_3	F/F	J/-	L/-	Z/-	H/-

[1]See Table I.
[2]Based upon results of progeny tests. See text.

TABLE III. Proportion of progeny from testcrosses that failed to inherit the blood group from its sire

TWIN SIRE	GENETIC SYSTEMS AND BLOOD GROUPS					
	B		C	L	Z	PROBABILITY ↓
	$BGKO_3A'$	BI_1	C_1X_1	L	Z	$(\frac{1}{2})^n$
362B		7/7		5/5	5/5	$(\frac{1}{2})^{17}=\ll.001$
362C	19/19		19/19			$(\frac{1}{2})^{38}\ll.001$

[2]The null hypothesis is that these blood groups are transmitted with a probability of ½, i.e., the sires are heterozygous.

ly, $1-(\frac{1}{2})^n$ gives the probability of the non-existence of these blood groups as heritable characters.

As shown in Table III, $p = (\frac{1}{2})^{17}$ for 362B and $P = (\frac{1}{2})^{38}$ for 362C. These probabilities are highly significant, being very much less than 0.001, and show that the acquired characters in the blood of each twin bull were not heritable.

The seven progeny of 362B and the 19 progeny of 362C possessed in expected proportions only those phenogroups corresponding to the genotype of the sire (Table IV). For example, seven of 19 progeny of 362C possessed QE_1I' and 12 possessed BI_1, giving a probability of greater than 0.1 that these blood groups behave as alternative alleles at the B system. Further, all 19 calves of 362C possessed the groups C_1X_3 of the C system as expected if the sire were homozygous for the causative genes. Similarly, the phenogroups $QE_1'I'$ and $BGKO_3A'$ of the B system of sire 362B segregated normally in a ratio of 2:5 among the seven progeny, as did the groups of the C system in a ratio of 4:3. Both of the sires were homozygous F/F, and all of their progeny possessed F as expected. The proportions of calves possessing the groups J, L, Z and H′ were in agreement with those expected when the blood types of the dams were considered. Thus, the genes controlling the blood groups of the respective twin bulls segregated as expected of individuals without erythrocyte mosaicism.

Discussion

The evidence presented shows that the intimate association, beginning in early embryonic life, of the two kinds of hematopoietic tissues in each twin did not demonstrably affect either the blood groups (cellular antigens) formed by these different tissues, or the genes for the cellular antigens transmitted by each twin to its offspring.

In the course of studies on bovine twins we have observed several situations similar to that described in this report. It is, of course, possible to study the progeny of males only from twins of unlike sex since the female twins showing erythrocyte mosaicism are sterile (freemartins). (It is interesting to note that the females with erythrocyte mosaicism in unlike-sexed twins in man have produced children[6, 12] in contrast to the well-known infertility of cattle[13, 17].) One bull twin with mosaicism produced 17 offspring, none of which inherited any of three independent blood groups which were apparently present somatically but not germinally in his blood. The probability of this occurrence if the bull actually possessed these factors genetically is $(\frac{1}{2})^{51}$. We have also observed the lack of transmission of characters shown phenotypically by four female twin pairs with mosaicism, representing a total of 23 offspring from all the matings. In one pair of female twins the proportion of the two cell types was 97:3 in each twin. Even though only three percent of the volume of erythrocytes of one twin was of her own genotype, she nevertheless transmitted this genotype detectably unaltered to each of five calves.

TABLE IV. Blood groups inherited by the progeny of the twin bulls

BULL TWIN	NO. OF PROGENY	GENETIC SYSTEMS AND BLOOD GROUPS										
		B			C		F	J	L	Z	H′
		$QE_1'I'$	$BGKO_3A'$	BI_1	C_1X_1	C_1X_3	F	J	L	Z	H′
362B	7	2	5	0	4	3	7	2	3	5	7
362C	19	7	0	12	0	19	19	9	10	10	14

It is interesting to speculate on the significance of these findings as regards the disreputed theory of the inheritance of acquired characters. According to Owen[13], ". . . since the interchange of formed erythrocytes alone between embryos could be expected to result in only a transient modification of the variety of circulating cells, it is further indicated that the critical interchange is of embryonal cells ancestral to the erythrocytes. . . ." A more intimate or long-lasting association of genetically distinct tissues than obtains in twins of cattle with erythrocyte mosaicism is practically impossible to visualize. Were there any effect of the foreign somatic cells and tissues upon either the phenotype or the genotype of the host, one would expect such an effect to be evident in these animals. None was observed on either the somatic or germinal cells of the host animals considered in this report, nor have any been reported by other workers who have studied cattle twins.

If embryos or newborn are subjected to foreign cells or tissues, the condition known as *actively acquired tolerance*[3] often results, whereas adults injected with foreign cells or tissues will be expected to show an immune response. Twins which show erythrocyte mosaicism are obviously "tolerant" to the antigens of the co-twin's cells and presumably to other tissues. It is apparent from the present results that there were no detectable changes in the germ plasm of either twin. It seems unlikely, therefore, that the "alterations" induced by foreign cells in producing actively acquired tolerance are reflected in the germ plasm.

Recent reports[8, 21] have appeared of changes in various characteristics either of the recipient animals or of their progeny, or both, following the injection of blood from the same species or

from a different species of animal. Generally these tests were done on White Leghorn chickens. References were also given[8] to reports of changes in other species on the part of the recipient or its progeny, or both, following contact with tissues from other breeds, by parabiosis, tissue transplantation, etc. In essence, these workers claim to have produced an effect transmittible to the progeny of treated individuals—therefore an effect on their germinal tissues. Professor J. F. Crow has kindly advised us of a paper by Galton[7] which is contrary to the claims of these authors[8, 21].

One may state that if the claims of these authors[8, 21] are verified, the task of population geneticists dealing with human material will be further complicated as a result of the widespread use of blood transfusion! In our opinion, however, the autonomy of the germ plasm remains unchallenged.

Summary

Blood typing of the progeny of a pair of male cattle twins with erythrocyte mosaicism showed that the intimate association, beginning in early embryonic life, of the two kinds of hematopoietic tissues in each twin did not demonstrably affect either the cellular antigens formed by these tissues, or the genes for the cellular antigens transmitted by each twin to its offspring.

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STUDIES ON THE MOUSE HEMOGLOBIN LOCUS

I. Identification of Hemoglobin Types and Linkage of Hemoglobin with Albinism

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DOUGLAS *et al.*[3] concluded from differences in the relative affinity of mouse hemoglobin for oxygen and carbon monoxide that the constitution of the globin moiety of hemoglobin varied among non-inbred mice. Anson *et al.*[1] confirmed their observations by further spectroscopic studies. Additional, more direct, evidence in support of their conclusions has been provided by electrophoretic analyses[4, 7-11, 13]. The inheritance of mouse hemoglobins has been partially characterized[4, 6, 9]. Linkage of the hemoglobin and albinism loci in linkage group I is reported in this paper, and the inheritance of hemoglobins is further discussed.

Linkage of Hemoglobin and Albinism Loci

Mice used in this investigation came from inbred strains maintained by the Drs. W. L. and Liane B. Russell, Biology Division of the Oak Ridge National Laboratory. Hemoglobin types were determined by electrophoresis. Samples of whole blood were obtained from severed tail veins, placed on small pieces (22 × 60 mm) of Whatman No. 1 chromatography paper, and analyzed by electrophoresis in starch gels, which were prepared with 0.03 *M* borate buffer at pH 8.48[12]. Since the hemoglobin patterns are most distinct at the center, the starch-gel blocks were sliced horizontally into upper and lower halves to facilitate examination. Hemoglobins were classifiable as either "diffuse" or "single" on the basis of their patterns in the gels with or without staining by amido Schwarz 10B[7].

Single-type hemoglobins were found in C57BL/6, C57L, C57BR, C57BL/10-D2, NB, and SeC mice. Diffuse-type hemoglobins were found in C3H, DBA/2, BALB/c, SeAB, CFW, CFCW, 101, 129, and FU mice.

Strains NB ($p\ c^{ch}$ with single-type hemoglobin) and SeAB ($P\ C$ with diffuse-type hemoglobin) mice were mated to obtain animals crossed to $p\ c^{ch}$ mice with single-type hemoglobin for coupling analyses. Similarly, F_1 progeny of BALB/c, CFW, or FU (with diffuse-type hemoglobin) and C57BL/6 (with single-type hemoglobin) mice were crossed to $p\ c^{ch}$ mice with single-type hemoglobin for repulsion analyses. A total of 1065 progeny were classified for p, c^{ch}, and hemoglobin traits (Table I). The three-point coupling data show that the hemoglobin locus lies outside the p-c segment of linkage group I; the linear order of the loci is p-c-hemoglobin. Combined analyses for coupling and repulsion show that the recombination frequency between c and hemoglobin is 2.49 ± 0.52 in males and 5.13 ± 0.58 in females. Differences in crossing-over between male and female mice have been observed previously in linkage group I, as well as in other linkage groups[5]. In the coupling analysis with females as heterozygotes (Table I), the numbers of $\frac{p\ c^{ch}\ Hb^a}{P\ c^{ch}\ Hb^a}$ and

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$\frac{p\ C\ Hb^d}{p\ c^{ch}\ Hb^s}$ progeny were somewhat larger and smaller, respectively, than would have been expected by chance. These results remain unexplained, but they probably do not affect the recombination values for the *c*-hemoglobin segment. It will be interesting to determine the linear order of *c*, hemoglobin, and *sh-1* since the existing data suggest that the hemoglobin locus lies inside the *c-sh-1* segment in males and outside in females.

Further Differentiation of Hemoglobin Types by Solubility and Crystallization

Our results from electrophoresis of hemoglobins of F_1 hybrids agree with data of Gluecksohn-Waelsch *et al.*[4]: matings of homozygous-diffuse by homozygous-diffuse gave diffuse-type, single by single gave single-type, and homozygous-diffuse by single gave diffuse-type hemoglobins. It could not be established by electrophoretic methods whether dominance or co-dominance existed in heterozygotes.

Other physicochemical properties of mouse hemoglobins were studied with the use of Derrin's techniques[2] for salting out of proteins in K_2HPO_4-KH_2PO_4 buffer. The results from use of this technique [which is useful for genetic analyses since it makes it possible to classify the heterozygotes since mouse hemoglobins can be identified by their solubility (Figure 8) and by the crystals they form in K_2HPO_4-KH_2PO_4 buffer[6]] suggest that heterozygotes have the single and the diffuse types in approximately equal quantities. Furthermore, differences in electrophoretic mobility in starch gel at different pH levels suggest that the single hemoglobin of C57BL mice is not identical to any fraction of the diffuse hemoglobin of 101 mice[8]. That these hemoglobins are not isomorphic is also indicated by differences in solubility and in the crystals formed in phosphate buffer. Results of alkaline denaturation of mouse hemoglobins imply that none of the hemoglobin genes is dominant[13]. Moreover, the expected 1:2:1 ratio was obtained when hemoglobins from progeny of F_1 intercrosses were analyzed by their solubility properties.

Several investigations[7,10,11,13] revealed that at least two electrophoretically separable fractions exist in diffuse hemoglobins; thus the locus that controls synthesis of diffuse hemoglobin may be a compound locus. We have observed minor hemoglobin fractions with rela-

TABLE I. Backcross progeny classified for pink-eye, chinchilla, and hemoglobin traits

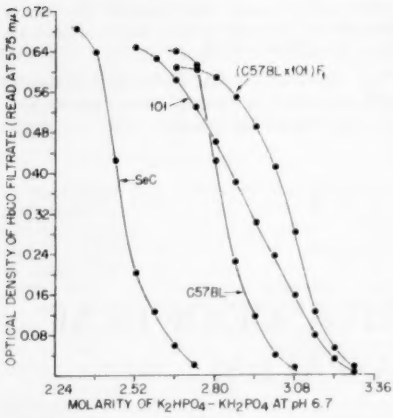
Genotype of progeny*	Coupling: $\frac{p\ c^{ch}\ Hb^s}{p\ c^{ch}\ Hb^s}$ ♀♀ × $\frac{P\ C\ Hb^d}{p\ c^{ch}\ Hb^s}$ ♂♂ — Number	Coupling: $\frac{P\ C\ Hb^d}{p\ c^{ch}\ Hb^s}$ ♀♀ × $\frac{p\ c^{ch}\ Hb^s}{p\ c^{ch}\ Hb^s}$ ♂♂ — Number
$\frac{p\ c^{ch}\ Hb^s}{p\ c^{ch}\ Hb^s}$	151	170
$\frac{P\ C\ Hb^d}{p\ c^{ch}\ Hb^s}$	139	167
$\frac{p\ C\ Hb^d}{p\ c^{ch}\ Hb^s}$	22	27
$\frac{P\ c^{ch}\ Hb^s}{p\ c^{ch}\ Hb^s}$	25	49
$\frac{p\ c^{ch}\ Hb^d}{p\ c^{ch}\ Hb^s}$	4	13
$\frac{P\ C\ Hb^s}{p\ c^{ch}\ Hb^s}$	4	12
$\frac{p\ C\ Hb^s}{p\ c^{ch}\ Hb^s}$	1	0
$\frac{P\ c^{ch}\ Hb^d}{p\ c^{ch}\ Hb^s}$	0	0
Total	346	438

Genotype of progeny	Repulsion: $\frac{p\ c^{ch}\ Hb^s}{p\ c^{ch}\ Hb^s}$ ♀♀ × $\frac{P\ c\ Hb^d}{P\ C\ Hb^s}$ ♂♂ — Number	Repulsion: $\frac{P\ c\ Hb^d}{P\ C\ Hb^s}$ ♀♀ × $\frac{p\ c^{ch}\ Hb^s}{p\ c^{ch}\ Hb^s}$ ♂♂ — Number
$\frac{P\ c\ Hb^d}{p\ c^{ch}\ Hb^s}$	24	120
$\frac{P\ C\ Hb^s}{p\ c^{ch}\ Hb^s}$	31	96
$\frac{P\ c\ Hb^s}{p\ c^{ch}\ Hb^s}$	0	6
$\frac{P\ C\ Hb^d}{p\ c^{ch}\ Hb^s}$	1	3
Total	56	225

Recombination values ± S.E.

♂♂ *p* to c^{ch} = 48/346 = 0.1387 ± 0.0125
♂♂ c^{ch} to *Hb* = 9/346 = 0.0260 ± 0.0058
♀♀ *p* to c^{ch} = 76/438 = 0.1735 ± 0.0122
♀♀ c^{ch} to *Hb* = 25/438 = 0.0571 ± 0.0075
♂♂ *c* to *Hb* = 1/56 = 0.0179 ± 0.0119
♀♀ *c* to *Hb* = 9/225 = 0.0400 ± 0.0088

*Symbols Hb^s and Hb^d indicate electrophoretically single and diffuse hemoglobins, respectively.



SALTING-OUT CURVES FOR HbCO FROM DIFFERENT MOUSE STRAINS

Figure 8

The optical density of the HbCO filtrate is plotted against the molarity of phosphate. Readings were made at 21 hours, and the physical conditions were 30° C and pH 6.7.

tively low electrophoretic mobilities, such as were reported by Rosa *et al.*[10], but the inheritance of these fractions was not investigated in this study. During ultracentrifugation, two sedimentation peaks were observed with hemoglobin preparations from red cells of strain 101 mice and one peak for C57BL mouse hemoglobin (R. A. Popp and R. E. Canning, unpublished data). Before two or more hemoglobin loci can be established, however, crossing-over within what is presently considered to be the locus must be demonstrated.

Our studies show that single-type hemoglobins of C57BL and SeC mice have very different solubilities in phosphate buffer (Figure 8) although they are similar in electrophoretic mobility and hemoglobin pattern. This observation suggests that a series of multiple alleles may eventually be identified at the hemoglobin locus or there may be interactions between the hemoglobin genes and those of other loci.

Hemoglobin Types in a Wild Population of Mice

Eighteen wild mice were collected in Karns Community, Tennessee, where matings with mice that might have escaped from the laboratory should be negligible. Six of these had single-type hemoglobin, three had homozygous-diffuse, seven had heterozygous-diffuse, and the other two had diffuse hemoglobin of undetermined genotype.

The gene frequency of single-type hemoglobin (0.57, based on the percentage of wild mice with single hemoglobin) is apparently higher in the group of wild mice we examined than in the laboratory mice[11]. A much larger number of wild mice must be examined to establish whether the 18 wild mice we sampled were indeed representative of the total population; however, it would seem that the selection coefficient for either type of mouse hemoglobin is probably quite small if, indeed, natural selection for one hemoglobin type is occurring.

Summary

The mouse hemoglobin locus is closely linked to albinism and on the opposite side of it from pink-eye. In mice heterozygous for different hemoglobin types, each hemoglobin type is present autonomously. This indicates that the inheritance of hemoglobin traits in mice may be similar to that in man. Differences among single-type hemoglobins have been reported, but whether these variations are controlled by different hemoglobin alleles or are caused by interactions of the hemoglobin locus with other loci remains to be established. The occurrence of different hemoglobin types in a small sample from a population of wild mice was also noted.

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SINGLE GENE INHERITANCE OF LEAF COLOR IN PASPALUM

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PASPALUM FLORIDANUM Michx. is an erect, robust, perennial grass occurring in low moist sandy soils, pine woods, savannas, and the low prairies in the Coastal Plain south of Maryland and Missouri[3]. *Paspalum floridanum glabratum* Engelm. grows in the same habitat but is more robust and taller. Extreme specimens of these species overlap in several morphological characteristics and especially as to combinations of size and pubescence[2]. An extreme which is characteristic of each species, however, is the dark green (Bice) color of *P. floridanum* as compared to the whitish green (Mytho) color of *P. f. glabratum*.

Observations made through the flatwoods of Mississippi have shown that numbers of *P. f. glabratum* were larger than the numbers of *P. floridanum* in mixed populations. This suggested a dominance of the *P. f. glabratum* type.

To explore the color differences of the two species led to the studies described here.

Materials and Methods

Representative plants of the two species were placed in three gallon pots for ease of handling. Crosses were made between the two species by means of hand emasculation and pollination. The dark green *floridanum* was used as the female parent. Selfed seed were obtained by enclosing panicles in manila bags just before flowering. Selfed progeny seed were germinated in sterile sand and transferred to individual pots. These were transplanted to the field and spaced six feet apart. Visual color determinations were made on each plant by means of color charts[5]. Inasmuch as the data concern only one degree of freedom, the chi-square value was adjusted for continuity according to Snedecor[7].

Results

The F_1 was completely dominant for the whitish green of the *P. f. glabratum*. No hybrid vigor was shown by the F_1 plants. In the F_2, 100 whitish green and 39 dark green plants were obtained. This is a satisfactory fit to a 3:1 ratio and indicates that the color difference of the two species is due to a single gene.

All the 39 dark green F_2 plants produced like progeny in the F_3 which

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shows the dark green color to be recessive to the whitish green. The whitish green F_2 plants produced 1344 whitish green and 288 dark green progeny. This is a satisfactory fit to a 5:1 ratio. The details of these data are shown in Table I.

Discussion

The relative ease with which the two species were hybridized, the fertility of the F_1 and F_2, and the segregation of progeny would indicate that *P. f. glabratum* possesses the same number of chromosomes ($2n = 160$) as has been reported for *P. floridanum*[1]. These also would suggest that the same genomes are present in both species. The absence of hybrid vigor in the F_1 tends to rule out major chromosomal differences between the species.

It is worthy of note that this single gene difference for color exists in species classed as sixteenploids, and that a character such as color would be simply inherited strongly indicates that they are allopolyploids.

That *P. floridanum* was first definitely classified taxonomically in 1803 and *P. f. glabratum* in 1886[2] would lead to the supposition that the latter species is of more recent origin and possibly could have originated as a mutation from the first described species. That the extremes of overlapping morphology are so striking and are relatively so numerous[2] and that the color difference is so definite adds weight to this hypothesis. On the other hand, the priority of species discovery and naming could be subjected to much philosophizing and in view of the data herein presented, *P. f. glabratum* might be considered as only a genetic type within the species *floridanum*.

The direct origin by mutation of one form from another is more likely if there is a single gene difference between the two forms than if there is more than a single gene difference. The data presented would indicate that this is the case with the color difference of these species.

It is generally agreed that recessive mutations occur much more frequently than dominant mutations[4, 6] and that most recessive mutations are unfavorable in their effect. While it is proposed that the color difference exhibited by *P. f. glabratum* arose as a gene mutation from recessive dark (Bice) green to dominant whitish (Mytho) green, the dominant mutation does not represent much of an improvement. However, being more robust[2] generally and with a slightly larger range of habitat[3], it possibly could be considered as a slight improvement.

Neither species is of any economic importance in agriculture. The huge ergot sclerotia formed in the florets of both species demonstrates that neither species can be considered as a source of genes for ergot resistance in a breeding program.

The overlapping of the range of morphological characteristics emphasizes that more detailed studies should be made.

Summary

The inheritance of color types of *Paspalum floridanum* and *Paspalum floridanum glabratum* as a single gene difference is described. The recorded taxonomic history of the two species indicates that *P. f. glabratum* is of more recent origin than *P. floridanum*.

That the color of *P. f. glabratum* arose as a dominant mutation from *P. floridanum* is suggested.

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TABLE I. Segregation of progeny of a *P. floridanum* × *P. f. glabratum* hybrid

Generation	a	t	a-t	$(a\text{-}t)-\frac{1}{2}$	$((a\text{-}t)-\frac{1}{2})^2$	$((a\text{-}t)-\frac{1}{2})^2/t$	χ^2
F_1				Complete dominance			
F_2	101	105	−4	3.5	12.25	12.25/105	.117
	39	35	4	3.5	12.25	12.25/35	.350
							.467 P = .50 — .25
F_3	1344	1360	−16	−15.5	240.25	240.25/1360	.1767
	288	272	16	15.5	240.25	240.25/272	.8833
							1.0600 P= .50 — .25

INHERITANCE OF PAPERSHELL CAPSULES, CAPSULE NUMBER AND PLANT COLOR

In Sesame

THOMAS W. CULP*

SINCE the discovery of the first indehiscent sesame mutant by Langham[2], breeding emphasis has been placed primarily on the development of varieties adapted to mechanical harvest. Considerable progress has been made toward developing varieties which do not dehisce; however, most of these are difficult to thresh. The utilization of the character known as "papershell capsules" may be helpful in overcoming this problem.

Papershell capsules are much easier to thresh than normal type. It was assumed that papershell capsules possessed very thin or "papery" walls in contrast to the walls of normal capsules. No such striking morphological differences have been observed in preliminary studies to indicate that this is the case. Further investigations will be necessary to determine the mechanism that is responsible for ease in threshing of the capsules. Since papershell capsules are easier to thresh, this character in combination with indehiscence promises to improve mechanical harvesting of sesame markedly.

Information pertaining to the inheritance of papershell capsules is very limited. The present study was undertaken to determine the inheritance of this character and to establish any possible relationships between capsule types, capsule number per leaf axil and plant color.

Materials and Methods

Three sesame crosses, T55249 (T53178-1-8-6-2-B × V54.11-2-B), T55250 (T53178-1-8-11-2-B × V54.11-1-B) and T55268 (V54.116-1-2-B × T53178-2-10-3-3-3), were used for this study. The T53178 parent possessed normal capsules, three capsules per leaf axil and green plant color. The V54.116 parent possessed papershell capsules, one capsule per leaf axil and purple plant color. The V54.11 parent possessed papershell capsules, one capsule per leaf axil and green plant color. (The V54.116 and V54.11 parents were obtained from D. G. Langham, Maracay, Venezuela.) Seed of the F_1, F_2 and backcrosses to both parents were produced in the greenhouse in 1955. The respective parents, crosses and generations were grown on the College Plantation of the Texas Agricultural Experiment Station, College Station, Texas, in 1956.

TABLE I. Observed segregation of capsule type and goodness of fit to monogenic and digenic F_2 and backcross ratios in three sesame crosses

Cross number	Generation	Phenotype		Expected ratio	Chi-square	P value
		Normal	Papershell			
T55249	F_2	137	50	3:1	0.301	0.5-0.7
	BC	34	32	1:1	0.061	0.8-0.9
T55250	F_2	140	41	3:1	0.532	0.3-0.5
	BC	41	32	1:1	1.110	0.2-0.3
T55268	F_2	88	78	9:7	0.697	0.3-0.5
	BC	26	49	1:3	3.737	0.05-0.1

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Results and Discussion

The observed segregation of capsule types in the F_2 and the backcross to the papershell parent in the three sesame crosses studied are presented in Table I. The data from the crosses T55249 and T55250 indicate that the papershell character is inherited as a simple recessive. Phenotypic segregations in the F_2 were very close to the expected 3:1 ratio. The pooled chi-square, 0.014, had a P value of .90 to .95. The backcrosses to the papershell parents approached the expected 1:1 ratio. The pooled chi-square, 0.870, had a P value of .3 to .5. The data from the cross T55268 indicate that the expression of the papershell character is the result of duplicate recessive epistasis. In the F_2 generation, 88 normal to 78 papershell progeny were observed. This agrees very closely with an expected 9:7 ratio in the F_2, assuming that duplicate recessive epistasis is in effect. In the backcross to the papershell parent, 49 papershell to 26 normals were produced. Although this is not a good fit, it approaches the expected 3:1 ratio.

The degree of intensity in expression of the papershell character varied considerably in these progenies. Some of them were very difficult to classify. In spite of the difficulty in classification and the relatively small number of plants under study, it is reasonable to assume that the inheritance of papershell capsule is controlled by two recessive pairs of Mendelian factors with duplicate recessive epistasis in effect. The two crosses T55249 and T55250 were apparently segregating for only one of these two genes. The symbol "p_1 p_1 p_2 p_2" is suggested as the designation for the papershell factors. The genotypes of the three parents would be represented as follows: V54.116 = $p_1p_1p_2p_2$, V54.11 = $P_1P_1p_2p_2$, and T53178 = $P_1P_1P_2P_2$.

The possibility that the papershell character is controlled by one recessive factor is not disproved. It is difficult to distinguish with certainty a 3:1 ratio from a 9:7 ratio, when a small progeny is analyzed. Additional studies should be undertaken with special emphasis on determining the inheritance of the degree of expression of this character and to deter-

TABLE II. Observed segregation of capsule number per leaf axil and goodness of fit to monogenic F_2 and backcross ratios in three sesame crosses

Cross number	Generation	Number capsules/axil		Expected ratio	Chi-square	P value
		One	Three			
T55249	F_2	147	40	3:1	1.318	0.2-0.3
	BC	20	27	1:1	1.042	0.3-0.5
T55250	F_2	142	39	3:1	1.151	0.2-0.3
	BC	21	14	1:1	1.400	0.2-0.3
T55268	F_2	132	35	3:1	1.456	0.2-0.3
	BC	13	13	1:1	0.000	0.99
All crosses	F_2	421	114	3:1	3.894	0.02-0.05
	BC	54	54	1:1	0.000	0.99

TABLE III. Observed segregation of plant color and goodness of fit to monogenic F_2 and backcross ratios in the sesame cross T55268

Generation	Plant color		Expected ratio	Chi-square	P value
	Green	Purple			
F_2	120	46	3:1	0.651	0.3-0.5
BC	2	74	1:1	68.20	0.0-0.0

TABLE IV. Linkage chi-squares between all possible combinations of characters in the F_2 generations of three sesame crosses

Characters	T55249		T55250		T55268	
	Chi-square	P value	Chi-square	P value	Chi-square	P value
Capsule type vs. capsule number	0.348	0.7-0.5	0.512	0.5-0.3	0.715	0.5-0.3
Capsule type vs. plant color	....	....	....	....	1.628	0.3-0.2
Capsule number vs. plant color	....	....	....	....	0.003	0.98-0.9

mine whether plants which carry both pairs of factors for papershell capsules thresh easier than plants which carry only one pair of these genes.

The observed segregation of capsule number per leaf axil in the F_2 and the backcrosses to the recessive parents in the three sesame crosses (Table II) indicate that one capsule per leaf axil is dominant over three capsules per leaf axil and controlled by one pair of Mendelian factors. These data confirm previous reports[1, 4]. Langham suggested the symbol "*tt*" as a designation of the genes for multiple capsules per leaf axil[1].

The observed segregations for plant color in the F_2 and in the backcross to the purple parent in the sesame cross T55268 (Table III) indicate that green is dominant to purple and that the expression of this character is controlled by one pair of genes. In the F_2, 120 green to 46 purple plants were observed. This approximates a 3:1 ratio. The segregation in the backcross to the purple parent did not approach the expected 1:1 ratio (chi-square = 68.20); two green to 74 purple plants were observed. This poor fit is difficult to explain but apparently selfed seed were obtained from the purple parent rather than backcross seed since a preponderance of purple segregates was observed. These data do not agree with a previous report by Langham[3], who found green to be recessive to purple and bronze. It is possible that different color characters were being investigated in the two studies. The symbol "*gg*" is suggested as a designation of the genes for purple.

Linkage or interaction chi-squares between characters in the three sesame crosses are given in Table IV. There were no indications of linkage between any of the three characters under study. Apparently selection for any one character will have no effect on the other two.

Summary and Conclusions

The phenotypes of the progenies in two sesame crosses indicated that papershell capsule is inherited as a simple recessive. One cross indicated that this character is the result of duplicate recessive epistasis.

The inheritance of capsule number was confirmed as being controlled by one pair of Mendelian factors with one capsule per leaf axil dominant over multiple capsules per leaf axil.

Plant color was found to be simply inherited as a single factor with green dominant over purple.

No interactions or linkages were found between the three characters: capsule type, capsule number per leaf axil, and plant color.

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